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A STUDY OF CALF RESPONSE TO AMMONIA AND HYDROGEN SULFIDE GASES

by

GLENN ALLAN NORDSTROM

(C)

A THESIS

SUBMITTED TO THE FACULTY OF GRADUATE STUDIES AND RESEARCH

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled "A Study of Calf Response to Ammonia and Hydrogen Sulfide Gases" submitted by Glenn Allan Nordstrom in partial fulfilment of the requirements for the degree of Master of Science.

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Date 30th July, 1975

ABSTRACT

With the recent trend towards total confinement production utilizing liquid manure systems, air environment has become a potentially critical element in livestock-housing design. While manure gas poisonings of cattle have been attributed mainly to hydrogen sulfide (H_2S), possibly in combination with ammonia (NH_3), almost nothing definitive is known about the effects of exposure to sub-lethal concentrations of these gaseous contaminants over the short or long term.

The study reported herein was undertaken to investigate the response of calves subjected to controlled levels of H_2S and NH_3, alone and in combination. Thirty-six steer calves averaging 474 lb liveweight were exposed to the various gas treatments in enclosed chambers for a continuous period of seven days. Data also were collected for seven day pre-exposure and post-exposure periods. The following results were obtained and conclusions drawn.

1. The most prominent clinical symptom of exposure to both H_2S and NH_3 was eye irritation. While the effects of NH_3 at 65 or 150 ppm were no longer obvious by the latter days of gassing, exposure to 20 ppm H_2S for one week apparently caused permanent tissue damage to the cornea. At 150 ppm, H_2S induced severe corneal opacity and rupture of the eye appeared possible toward the end of the gas-exposure period. Epistaxis was a prominent feature of exposure to 150 ppm H_2S, especially in combination with 150 ppm NH_3.
2. Ammonia at 65 or 150 ppm had no appreciable adverse effect on average feed and water consumption during the gas-exposure period. Hydrogen sulfide alone at 20 and 150 ppm reduced mean

weekly feed consumptions by 3.5 and 26% respectively. Although 20 ppm H_2S did not affect mean water intake, at 150 ppm H_2S water intake was restricted to 75% of normal over the exposure period. The effects of H_2S and NH_3 in combination on feed and water consumption appeared to be non-interactive.

3. Although respiratory frequencies appeared to be slightly increased at the low levels of H_2S (20 ppm) and NH_3 (65 ppm), and slightly decreased at the high concentrations (150 ppm) of H_2S and NH_3, respiration rate was not regarded as a reliable indication of the effects of the gases on the respiratory system.

4. Rectal temperatures were affected similarly by most treatments in that temperatures were highest near or on the fourth day of gassing but approached normal by the last day of exposure. Mean period rectal temperatures were elevated by exposure to 150 ppm NH_3 alone, and to 150 ppm H_2S alone and in combination with 65 and 150 ppm NH_3.

5. Tests to detect sulfhemoglobin in the blood were negative for all calves. Extensive measurements of other blood constituents generally were inconclusive and hence failed to reveal any hematological values that may have been correlated with the toxicity of H_2S and NH_3.

6. The detrimental effects of exposure to sub-lethal levels of H_2S and NH_3 appeared to be related more to gas concentration than to length of exposure time. Indications were that the action of H_2S was more severe and extensive than that of NH_3, while the effects of the two gases in combination usually

appeared to be additive; that is, one gas did not intensify the action of the other.

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I. INTRODUCTION

In efforts to maximize production efficiency there has been a decided acceleration in confinement housing of cattle in North America during recent years. This specialization has resulted in concentrated production of manure and consequent problems of disposal. Handling manure as a fluid offers the advantages of lower labor requirements and greater adaptability to confinement systems of livestock production. As a result, slatted-floor free-stall barns and regular-stall barns with manure storage beneath are in common use in milk production operations, and dairy calves are being raised on slatted floors in increasing numbers (13). Also, in the beef industry, a growing number of feeders are being attracted to confinement systems incorporating slatted floors over liquid manure pits (51,68,86,113). The limited duration of the pasture period in Canada would suggest that total confinement should have particular merit in this country (29).

Unfortunately, the storage of liquid manure within the animal quarters introduces specific problems (13,90): 1) toxic effects of manure gases on animals; 2) the stress produced on the unprotected animals resulting from radiant heat loss to the liquid surface or the floor; 3) odor control; and 4) potential damage to structural components of the confinement building. Perhaps the most dramatic and immediate concern is the effects of manure gases on animal health and performance.

Experiences with confined housing have shown that the gases evolved from manure can have detrimental effects on animals during at least two different extremes (58). The first main problem arises during cleaning operations of manure stored under slatted floors (120). Several

instances attributed to high levels of noxious gases can be cited where housed cattle have succumbed to severe sickness and many times death. Instances have been reported from Canada (68), Sweden (16,64,67), Germany (63,99) and the Netherlands (55). The conditions resulting in such accidents have generally occurred in connection with agitation of the liquid manure and/or under poor ventilation conditions (14,17,115).

The second problem arises when animals are exposed for prolonged periods to low levels of toxic gases practically encountered in an enclosure. Numerous reports (16,45,67) have suggested that such exposure could be detrimental to the productivity of cattle and at times create complications that are responsible for sickness and death.

The first problem of high concentrations of manure gases for a short period of time is recognized and easily rectified by either removing the animals from the shelter before the pits are cleaned out or by providing an adequate means of ventilation before waste removal begins. Levels of many of the gaseous products responsible for rapid death are known for man and other animals so that valid safety limits can be estimated (58).

The second problem of low concentrations is one of greater complexity. Almost nothing definitive is known about the long-term effects of slightly increased levels of manure gas normally found in cattle facilities. Exposure to higher but sub-lethal concentrations for short periods, such as may occur when slurry below slats is disturbed even where ventilation is assessed as good, may also affect the health of cattle.

As early as 1928, Hofmann (63) in Germany reported that gases from liquid manure could cause abortion in cows and that there was a

connection between manure gases and cases of respiratory diseases. In Sweden, a chronic manure gas poisoning of cattle was described for the first time in 1965 (16). The characteristics were a general deterioration in condition and production, respiratory and cardiovascular problems, tendencies to hemorrhage and eventual lameness. Further data regarding chronic manure gas poisonings in Sweden have been subsequently presented (64,66). A review by Hogsved and Holtenius (67) summarized much of the existing European literature on manure gas poisoning. These authors feared that, in Sweden, a large number of dairy cow herds were affected, in one way or another, by the poisonous gases from liquid manure. Apparently several cases of chronic poisoning have been so extreme that the effects from the point of view of production economy have been catastrophic.

In North America, studies (25) have shown that in recent years a number of dairymen have experienced a sudden loss of 50 to 60 per cent of their calves where previously losses were usually 5 per cent or less. In one of several cases cited by Brevick et al (25), when healthy calves were moved to pens on slats most of them soon developed respiratory and other problems and many died. Another incident (13) showed evidence, upon slaughter, of some lung damage in nearly all of 50 veal calves grown in individual metal stalls in a well insulated building where temperature and relative humidity were controlled through ventilation and the use of supplemental heat. Half of the calf stalls were located over a pit 10 feet deep and half were located over a shallow pit flushed daily into the deep pit. The examining veterinarian and an agricultural engineer agreed that lack of sufficient exhaust from the manure pit was

a contributing cause of insult to the lung tissue. One veterinarian (13) estimates that respiratory infection accounts for about 75 per cent of calf losses in his practice, and that 80 per cent of calf treatment is for respiratory-related disease.

Although adverse effects of manure gases appear to be well substantiated in the literature, the etiology of both acute and chronic poisonings has not yet been defined. Hydrogen sulfide (H_2S), a well known constituent of manure gas, generally has been presumed responsible for the numerous symptoms (16,17,64,68,99). However, quantification of these effects has been limited since most cases lacked proper controls and few estimations of the numerous gaseous compounds that might be responsible were determined (17). Suggestions have been made (55,67,97) that other gas components in addition to H_2S may play an important role in poisonings, notably the combination of H_2S and ammonia (NH_3).

"The effects of toxic gases such as ammonia, methane, carbon dioxide and hydrogen sulfide upon animal performance are not well defined. While a knowledge of lethal levels is important, it is more critical to determine the effects of prolonged exposure to sub-lethal or low levels. A higher incidence of respiratory disorders may result from low concentrations of toxic gases: the health of both livestock and humans may be jeopardized. The extent of the problem of toxic gases should be ascertained at a very early date...". These statements were included in the proceedings of a work planning meeting on dairy and beef cattle research sponsored by the Canada Department of Agriculture (29) in 1967.

Essentially the same words have been repeated by Hazen (59) in 1971 and again by Bates (13) in 1974. Although speculation abounds

regarding deleterious air-factor effects on livestock performance, too few specific data have been accumulated to permit formulation of design guidelines. This is presumably because high density confinement production utilizing liquid manure handling systems is a relatively new practice, and only with this trend has air environment become a potentially critical element in livestock enterprises.

In short, manure gases, particularly H_2S and NH_3, may affect performance of cattle but researchers have yet to generate definitive experimental evidence. The effects of acutely noxious levels of manure gases are recognized, but such high levels occur infrequently and are usually avoidable. To quote Curtis (37), the basic question is: "For each class of livestock are there effects on performance of continuous exposure for periods on the order of months to practically encountered levels and combinations of air factors?" An answer to this underlying question must precede definition of tolerance limits for use in cattle-housing design.

The major objectives of this study, therefore, were 1) by means of a review of the literature, to investigate the prevalent components of manure gas, their occurrence in cattle facilities, and the possible physiological symptoms and responses of humans and domestic animals exposed to these components; 2) to summarize and assess criteria that may be useful in evaluating and elucidating the response of cattle to the more toxic gases arising from anaerobic degradation of manure, namely H_2S and NH_3; and 3) on the basis of the findings of the literature review, to investigate the response of calves exposed to controlled levels of H_2S and NH_3, alone and in combination.

II. LITERATURE REVIEW

1. Noxious Gases Existing in Cattle Facilities.

1.1 Production of Gases.

The gases found in confinement buildings may arise from the livestock themselves, and from the bacterial decomposition of stored excreta.

1.1.1 Gases Produced by Livestock.

Extensive research has indicated that carbon dioxide (CO_2) and methane (CH_4) are the primary constituents of the vitiated air expired by ruminants. Carbon dioxide is the gas produced in greatest quantity. It is one of the by-products of animal energy metabolism and is expired via air exchange in the lungs. In addition to the CO_2 in the expired air, there is also an appreciable quantity produced by fermentation in the rumen, along with smaller quantities of CH_4. A cow may produce up to 50 litres of CH_4 per day while production of CO_2 may approach 300 litres per day (20). Much of these fermentation gases are voided by eructation (69). The remaining contaminant gases found within a livestock building are produced as a result of the decomposition of animal waste.

1.1.2 Gases Produced by Stored Wastes.

Oxygen from the air is excluded from wastes stored within a confined unit in the form of liquid manure. Under such conditions anaerobic bacteria degrade the organic and inorganic constituents of manure, producing gaseous emissions. Hydrogen sulfide, NH_3, CH_4 and CO_2 are some of the major gases which have been traced from cattle wastes (3,55,97,115). Chromatographic analysis of volatile substances over dairy waste (128) identified hydrogen sulfide, methanethiol, dimethyl

sulfide, diethyl sulfide, propylacetate, n-butyl acetate, trimethylamine and ethylamine. Dimethyl sulfide was considered the principal component of anaerobic dairy waste odor. Clark and Viessman (35) suggest that the main gaseous products of anaerobic digestion of domestic sewage are CO_2 and CH_4 with traces of H_2S and NH_3, and estimate that the total quantity of gases from one pound of volatile material is 0.56 - 0.64 cu ft (16 - 18 l). Cattle manure varies so widely that it is difficult to accurately predict properties. Estimates (121,124) of daily manure production of 1200 - 1500 lb (545 - 682 kg) dairy cows range from 88 lb (40 kg) to 99 lb (45 kg) of which about 67% is feces and 33% is urine. The mixture totals about 1.6 cu ft (45.3 l), weighs about 62 pcf (993 kg/cu m) and contains about 12-20% dry matter. Beef cattle and dairy heifers usually produce slightly drier manure; a growing beef animal weighing 700 - 1200 lb (318 - 545 kg) may produce 33 lb (15 kg) of feces plus 11 lb (5 kg) of urine daily, totalling 3/4 cu ft (21.2 l) and weighing about 60 pcf (961 kg/cu m) (121).

An important property to recognize is that when diluted by water to facilitate handling, liquid cattle manure in storage separates by gravity (121). Solid wastes having densities greater than water form a bottom sediment while lightweight particles float to the top. Between the two sludge layers the remaining manure is relatively fluid. Thus, thorough remixing is necessary before pits are emptied to prevent the fluid fraction from flowing out and the solids remaining. The adverse result of agitation is that it drastically enhances the release of gases from slurry.

1.1.3 Process of Anaerobic Degradation of Manure.

The bacterial degradation of anaerobically fermenting manure

has been described concisely by Barber and McQuitty (12). For simplicity, the organic portion of manure may be considered to be composed of proteins, carbohydrates and fats. In the absence of dissolved oxygen, anaerobic bacteria degrade the proteinaceous material to NH_3, H_2S and short-chain organic acids. The decomposition of carbohydrates proceeds initially with the production of organic acids which, together with the acids formed from proteins are converted further to alcohols or degraded to water, CO_2, and short-chain hydrocarbons, including CH_4. Fats are degraded to fatty acids and alcohols, with the ultimate decomposition of fatty acids to H_2O, CO_2 and CH_4. Alcohols produced either directly from fats or indirectly from proteins and carbohydrates are subject to oxidation reactions resulting in the formation of aldehydes and ketones. Furthermore, NH_3 can combine chemically, by displacement reactions, with alcohols and organic acids to produce amines and amides, respectively. Similarly, H_2S can react with alcohols and organic acids to produce mercaptans and thioacids, respectively. Reactions between sulfides (formed in aqueous solutions containing dissolved H_2S) and the short-chain hydrocarbons also are possible, yielding disulfides (e.g. dimethyl sulfide). One further reaction is that occurring between H_2S and CO_2 to yield carbonyl sulfide. In summary, the major gaseous emissions resulting from the anaerobic degradation of manure organics are NH_3, CO_2, H_2O, H_2S, CH_4 and other short-chain hydrocarbons, fatty acids, aldehydes and ketones, alcohols, amines, amides, mercaptans, disulfides, and carbonyl sulfide.

In addition to degradation of organic fractions of manure by heterotrophic bacteria, gases also may be produced from inorganic manure

constituents. The organisms responsible utilize oxidized inorganic compounds as electron acceptors in their energy metabolism (e.g. sulfate reduction to H_2S and nitrate reduction to NH_3 and nitrogen gas). They may use either organic or inorganic compounds as nutrient sources and as electron sources in their energy metabolism and, on this basis, are classified as heterotrophs or autotrophs, respectively. The major gases produced by these bacteria are CO_2, NH_3 and H_2S.

Some bioengineering parameters affecting gas production are temperature and pH (128). Both vaporization and biological activity related to the van't Hoff effect increase with temperature. Hydrogen-ion concentration influences the activity of microorganisms and enzymes. The optimum pH for amino acid decarboxylation is 4 to 5 with the release of amines and sulfur compounds. Deamination occurs at a high pH with the release of NH_3 and organic acids. At pH values of 8 and above, reduced sulfur exists mainly as sulfide ions (HS^-, $S^=$), but at pH 7 the formation of un-ionized H_2S is about 80% complete, so that the partial pressure of H_2S becomes great enough to cause gas problems (128).

1.2 Occurrence of Gases.

1.2.1 Properties and Distribution of Gases.

The composition by volume of dry, outdoor air is approximately 78.09% nitrogen, 20.95% oxygen, 0.93% argon and 0.03% carbon dioxide, with slight traces of inert gases (71). Owing to the many processes going on in livestock buildings, the composition of the air can become a complex assortment of chemical compounds. Some important properties of NH_3, H_2S, CO_2 and CH_4, prevalent gaseous products of manure decomposition (90,120), are summarized in Table 1.

Until recently, the concept of gases heavier than air

TABLE 1: RELEVANT PROPERTIES OF GASES*.
(@ 25°C, 760 mm Hg unless specified otherwise)

Gas	Formula	s.g.** (air=1)	Odor	Color	Affinity for water
Ammonia	NH_3	0.597	sharp, pungent	colorless	highly soluble (90g/100 ml H_2O@0°C)
Hydrogen Sulfide	H_2S	1.189	offensive (rotten egg)	colorless	moderately soluble (0.6g/100 ml H_2O@0°C)
Carbon Dioxide	CO_2	1.53	odorless	colorless	highly soluble
Methane	CH_4	0.554	odorless	colorless	slightly soluble

* compiled from references 14,58,71,87,88,101,120.
** Specific gravity.

accumulating near floor level and gases lighter than air accumulating at ceiling level has been used as a criterion in ventilation design. Taiganides and White (120) stated that CO_2 and H_2S, being heavier than air, would stay near the floor in a non-ventilated room while NH_3 and CH_4, being lighter than air, would rise from the point of generation and accumulate near the ceiling. However, decisive studies (24,52,97,107) have discounted the theory that gases tend to diffuse and accumulate in the atmosphere at different levels depending on their relative densities. In experiments conducted at constant temperatures with still air, Noren et al (97) found that heavier gases (CO_2, H_2S) and lighter gases (NH_3) which were released into a manure gutter, diffused fairly uniformly throughout the building. In measurements made under practical conditions in cattle stalls the concentration of CO_2 was found to be about twice as high by the ceiling as by the floor and in the gutter (97). This was

explained by the fact that this gas stems mainly from the air exhaled by the animals, which has a higher temperature than the air in the stall and therefore rises. Concentrations of NH_3 were distributed equally except below slatted floors where levels were somewhat higher than up in the stalls.

Noren et al (97) also observed that, in slatted-floor facilities, upward air currents from gutters emerged where the cattle were located and that downward currents occurred in areas free of animals. These observations confirm earlier reports of Ober (98) concerning air exchange through slatted floors and have recently been substantiated by Bruce (27). Noren et al (97) attributed the air currents to the heat production of the animals and, in agreement, Brannigan and McQuitty (24) reported that sensible heat was a major factor in the diffusion of gases in the atmosphere. Bruce (27) has said, "So far as animals lying on slatted floors are concerned the implication is clear." He deduced that unless forced convection is dominant, however air passes down through the floor and wherever it rises, the air eventually will move towards the animals as they are the sources of heat. Air currents must carry with them the gases produced from the slurry and thus any animals lying on a slatted floor would probably experience the maximum concentration of gas above the floor. Ventilation outlets below the floor may be beneficial in reducing gas concentration at animal level as was noted to a slight degree by Feddes and McQuitty (52) for NH_3.

1.2.2 Levels of Gases Measured in the Field.

Examinations of the air in animal shelters with liquid manure handling have been carried out primarily in poultry and swine buildings (9,40,47,76,77,80,81,84,90). Basic data have been sparse, however, on

the occurrence of gases in cattle stalls. The most complete investigation has been performed by the Swedish Institute of Agricultural Engineering during 1967-1971 (109). Under practical conditions, no measurable amounts ($\geq$1 ppm*) of H_2S were found in ventilation air as long as the manure remained undisturbed. This, however, does not exclude the possibility that H_2S can be emitted from manure continuously or in connection with changes in air pressure, temperature, or other occasions (97). With a ventilation rate of about 76 cfm per cow when manure lay still, CO_2 varied between 1500 and 3000 ppm while CH_4 varied between 150 and 300 ppm. Concentrations of NH_3 were measured at 5 to 10 ppm under similar conditions (97).

The same report noted that even a slight agitation of liquid manure can liberate poisonous gases and measured H_2S concentrations of 15 ppm at 1 to 2 metres above the slats during mixing operations. At times, H_2S levels exceeded 200 ppm in the gutter.

A survey by Haartsen (55) showed concentrations of H_2S, NH_3 and CO_2 to be nil, 30 ppm and 500 ppm respectively, over liquid cow dung prior to agitation. During agitation, measurements at animal level were: 120 to 600 ppm H_2S, 700 ppm NH_3, and 2000 ppm CO_2. Oxygen content of the atmosphere in the barn was only slightly below normal at 20.1% by volume.

During manure removal operations, Hudek (68) reported concentrations of H_2S ranging from 50 ppm to 250 ppm at animal level when the slurry was agitated, while concentrations did not exceed 5 ppm H_2S when the liquid was pumped from the pits without agitation.

Taiganides and White (120), in reviewing menacing concentrations

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* Parts per million by volume in air.

of noxious gases in animal units, have suggested that the normal range of gases originating within a cattle building will be about 0 to 20 ppm H_2S, 0 to 20 ppm NH_3 and 500 to 1000 ppm of CO_2, but that these concentrations can increase 5 to 10-fold when manure is stirred. Albin (3) states that levels of 1000 ppm H_2S are not uncommon when anaerobic pits are cleaned out.

2. Standards for Prevalent Gases Produced in Cattle Facilities.

2.1 Classification of Gases.

From the standpoint of respiration, gases may be classified as follows (60):

Group I-Irritants: Gases of this class injure the air passages or the lungs, or both, and induce inflammation of the surfaces of the respiratory tract (e.g. NH_3, H_2S at sub-acute levels).

Group II-Asphyxiants: (i) Simple asphyxiants: physiologically inert gases which, when breathed in high concentrations, act mechanically by excluding oxygen (e.g. CO_2, CH_4).

(ii) Chemical asphyxiants: substances which, by combining with the hemoglobin of the blood or acting upon some constituent of the tissues, either prevent oxygen from reaching the tissues or prevent the tissues from using it (e.g. CO_2).

Group III-Volatile Drugs and Drug-like Substances: These gases exert little or no specific effect upon the lungs; they act after they have been absorbed into the blood and transported to the tissues of the body (e.g. hydrocarbons).

Group IV-Inorganic and Organometallic Substances: This group includes a large number of poisonous elements and compounds occurring in volatile form and exerting a wide variety of toxic actions

after their absorption into the body (e.g. H_2S at acute levels).

2.2 Human Exposure.

Although pollutants of industrial origin primarily concern public health workers, air-factor limits for the major noxious gases found in livestock accomodation have been established for human workers.

Maximum admissible levels of H_2S have been given as 20 ppm in some references (14,81,101,120) and as 10 ppm in others (5,47,88,96,97). Similarly, maximum recommended levels for NH_3 have varied at 100 ppm (14,81,93,101,120), 50 ppm (47,87,97) and 25 ppm (5). The occupational health standards that are recognized as legislation by the Province of Alberta are the Threshold Limit Values (TLVs) of Airborne Contaminants published by the American Conference of Governmental Industrial Hygienists and its subsequent amendments or revisions (106). The levels of H_2S, NH_3 and CO_2 considered dangerous to man by this legal body are presented in Table 2. A TLV is not recommended for methane (CH_4) because it acts primarily as a simple asphyxiant. Thus the limiting factor is the available oxygen, which should be not less than 18% by volume under normal atmospheric pressure. The Governmental Industrial Hygienists (5) also have recommended that when two or more hazardous gases are present, and in the absence of information to the contrary, the effects of the different gases should be considered as additive; that is, if the sum of the following fractions,

$$\frac{C_1}{T_1} + \frac{C_2}{T_2} + \ldots + \frac{C_n}{T_n}$$ exceeds unity, then the TLV of the mixture should be considered as being exceeded. C indicates the observed atmospheric concentration, and T the corresponding TLV for each gas, n.

TABLE 2: OCCUPATIONAL HEALTH STANDARDS FOR GASES.

Gas	TLV[a] ppm	Excursion Factor[b]	TWA limit[c] ppm
H_2S	10*	2	20
NH_3	25**	1.5	37.5
CO_2	5000	1.25	6250

* Lowered from 20 ppm to 10 ppm in 1964.
** Lowered from 50 ppm to 25 ppm in 1974.

a TLV (threshold limit value) represents conditions under which it is believed that nearly all workers may be repeatedly exposed for an 8-hour day and 40-hour work week without adverse effect.

b Excursion factor defines the magnitude of the permissible excursion above the TLV.

c TWA (time-weighted average) limit defines the maximum concentration permitted for a short exposure period.

TLV x Excursion Factor = TWA limit.

2.3 Animal Exposure.

Until more information regarding the effects of manure gases on livestock is made available and subsequently accepted and applied, the only standards governing permissible levels in confinement buildings are those providing safe environments for workers. However, as mentioned earlier, human limits for the noxious gases have been established for a maximum 8-hour day, 40-hour week exposure period. In contrast, confined animals confront their environments almost continuously and may face many potentially harmful gases simultaneously. Moreover, confined livestock do not necessarily have the freedom to escape from irritating or hazardous situations. Clearly, important reservations accompany any application of human limits to livestock.

Henderson and Haggard (60) have warned that great caution must be exercised in drawing conclusions applicable to man by observing the effects of gases on animals. The converse also must hold. Therefore, except for demonstrating that certain concentrations are unquestionably fatal for short exposure, or that a gas is virtually non-toxic even in very high concentrations, there are two main factors limiting any broader application. First is a question of the qualitative effect on certain species - does the gas affect man in the same way that it does an animal? The second factor is that of quantity - if the gas does affect both in the same way, are they affected to the same degree?

In the opinion of Curtis (37), even inferences of animal response between different species are impermissible. However, others (80,81,84,120) have reasoned that animal responses are similar to those of humans, but vary in intensity with animal weight and time of exposure. Thus, effects found to occur in adult humans may be felt by animals weighing about 150 lb, especially pigs. Accordingly, cattle may be affected by noxious gases the same way as smaller animals such as poultry and swine, but for a given concentration the same physiological effect may not register as soon because of their size and body weight.

The limitations of correlating concentrations and physical response for gases have been discussed by Henderson and Haggard (60). The action of irritants (e.g. NH_3) in producing inflammation, causing death on short exposures, or being dangerous to breathe for any length of time is fundamentally the same in all animals and man. But effects on different animals from sub-acute concentrations cannot be quantitatively applied between species, and hence are valuable only as a guide.

Furthermore, prolonged exposures to certain irritants may result in a chronic respiratory disease - a point for which reliable quantitative information cannot be obtained from different species.

Inorganic gases (e.g. H_2S), aside from their irritation, exert an anesthetic action when inhaled in sufficient concentrations. Information on acute anesthetic effects can be applied directly to various species, but not when the anesthetic effects are slight. Mild intoxication may affect judgement - a function impossible to evaluate in animals. Special attention must be given to differences in species concerning the nature of chronic effects resulting from secondary toxic action which may appear only after prolonged or repeated exposures. To make any direct quantitative application under these conditions is virtually impossible.

With the simple asphyxiants (e.g. CO_2 and CH_4), the physiological responses can be generally applied to both man and animals with little modification. There is full knowledge on the response to various concentrations of asphyxiants; however, an uncertainty does lie in the question of alleged chronic functional disturbances from repeated exposures to concentrations too low to cause unconsciousness.

3. Physiological Action of Prevalent Manure Gases.

3.1 Asphyxiants.

Carbon dioxide and CH_4 are both simple asphyxiants and induce asphyxia entirely by excluding O_2 from the lungs, their effect being proportional to the extent to which their presence diminishes the partial pressure of O_2 in the expired air (60). They must be present in considerable amounts before they exert any appreciable effect, and may

decrease the O_2 of the air to two-thirds of its normal percentage (13% of an atmosphere) before noticeable symptoms of anoxemia develop (Table 3). At this concentration of asphyxiants (33% of air-gas mixture), the volume of breathing is increased and the pulse rate is accelerated. (Both these conditions actually begin earlier, but not noticeably.) When the O_2 is diminished to between 14 and 10 per cent, the higher centers of the brain are affected with subsequent loss of the sense of pain. Respiration is frequently intermittent, fainting may occur and muscular effort may permanently injure the heart. Below 10 per cent O_2 (50% gas), nausea and loss of movement are followed by loss of consciousness. When the O_2 is diminished below 6 per cent (75% gas), breathing stops (60).

TABLE 3: PHYSIOLOGICAL RESPONSE OF ADULT HUMANS TO CARBON DIOXIDE AND METHANE*.

Gas	Effect	Concentration (ppm)
CO_2	Safe	20,000
	Increased breathing	30,000
	Drowsiness, headaches	40,000
	Heavy, asphyxiating breathing	60,000
	Could be fatal (30 min exposure)	300,000
CH_4	Headache, non-toxic	500,000

* Taken from reference 120.

3.2 Irritants.

Since in concentrations lower than those causing marked systemic

effects NH_3 and H_2S both act as irritants, they have one physiological property in common (60): they induce inflammation in tissues with which they come in direct contact. Therefore, inflammation is manifest almost solely in surface tissues; that is, the skin, the conjunctiva of the eyes, and particularly the epithelium and mucous membranes of the respiratory tract.

The action of irritant gases has been explained by Henderson and Haggard (60). The inflammation process is a physiological reaction which is made by the tissues in response to a disturbance in the normal vital processes. To exert its action an irritant must be taken up by the surface tissues, and be dissolved in the fluid which bathes them. The locus of action on the respiratory tract is influenced by differences in the physical state of the gases, primarily their solubility. Thus, the concentration of NH_3 which reaches the lungs is greatly reduced by absorption in the upper passages. Because H_2S is relatively less soluble, its locus of action in the respiratory tract is much more extensive and thus H_2S is the more insidious in its action.

The severity of action of an irritant gas does not vary in proportion to the product of the amount of the gas in the air multiplied by the duration of exposure to it, as is the case with an asphyxiant gas. A high concentration for even a short time has an intense effect. Reducing the concentration by one-half allows an irritant to be withstood for much more than twice as long with less effect. Consequently, any reduction in concentration during passage through the upper respiratory tract results in more than proportionate sparing of the tissues of the lungs.

3.2.1 Irritation of the Respiratory Tract.

The delicacy of the respiratory membranes, their susceptibility to injury, and the seriousness of the damage which results from the action of irritant gases are very different in the upper and lower respiratory tracts. The first visible reaction to irritation of the upper respiratory tract is redness due to the dilation of small vessels in the affected area. This is accompanied by increased activity of the mucous glands in the surface membranes. Moderate exposure may not extend beyond this stage; the symptoms then are those of pharyngitis, laryngitis, and trachitis - pain, some swelling, redness and increased flow of mucus. If the irritation is severe, plasma may exude from the engorged blood vessels, producing marked swelling, separation of the tissues and their consequent destruction. This exudate also may block the respiratory passages.

Changes in the upper respiratory passages and bronchi first appear as a loss of normal gloss and translucency together with swelling. Plasma which exudes to the surface, clots there and forms a tenacious layer which is frequently streaked with extravasated blood. In drastic cases the mucosa may be lifted from the submucosa by the fluid and sloughed off, leaving raw and oozing surfaces covered with mucopurulent material. Before regeneration of the mucosa can occur, infection of the bronchi generally develops since the normal barrier to bacterial invasion is removed. The infectious organisms are the normal flora of the mouth and upper respiratory tract. Depending on the severity of inflammation, the symptoms resemble head cold, laryngitis, bronchitis, or bronchopneumonia. In exceptionally severe exposure, particularly to NH_3, the soft tissues about the larynx may become so swollen from the

extravasated fluid into the tissues that the trachea is occluded. Laryngeal edema causes death from acute suffocation.

The action of irritant gases upon the lungs results in pulmonary edema, a condition which reaches its height in from four to 24 hours after exposure. Fluid extravasates from the capillaries and accumulates between the tissue cells of the finer air-sacs in the lungs. Fluid also coagulates in the alveolar spaces and fills them with fibrin, thus seriously interferring with the respiratory exchange of O_2 and CO_2. At the same time, the flow of blood through the lungs is obstructed, placing a severe strain on the heart. Moreover, the loss of fluid from the blood in the form of exudate may deplete the body of water, decrease the volume of blood and increase its viscosity. Owing to bronchoconstriction as well as to the plugging of the bronchi with sloughed mucosa and fibrin, portions of the lungs may escape the action of the gas. Consequently, areas of atelectasis (collapsed or airless state of the alveoli) and emphysema (overinflated state of the alveoli) may occur in the edematous lung.

Irritation of the lungs does not give rise to severe pain as does inflammation of the upper passages, the principal symptoms being those of asphyxia. Peculiarly, in its early stages, the asphyxia is not associated with marked air hunger, and yet the condition can be acutely dangerous. This state, characterized in humans by gray cyanosis without marked dyspnea, is due to the unequal ventilation of the blood with respect to CO_2 and O_2. Since CO_2 is much more soluble and hence diffusable than O_2 in the plasma which covers the surface of the alveoli, CO_2 is eliminated more readily than O_2 is absorbed. At first, therefore, the O_2 of the blood is decreased without any appreciable rise of CO_2. There may even be a decrease of CO_2 owing to some degree of overbreathing, for although relatively slow in

developing, O_2 deficiency is a stimulant to respiration.

As the edema progresses, some of the alveoli become filled with frothy fluid and the terminal bronchioles become plugged. The air occluded within such areas develops the same gas pressures as the venous blood since they take little part in gaseous exchange. At the same time, less edematous areas are hyperaerated, causing the CO_2 level in the blood passing through them to be reduced below normal. However, due to the characteristics of the oxyhemoglobin dissociation curve, the O_2 content of the blood passing through these parts cannot be raised above the amount that the hemoglobin normally holds. Thus, of the pulmonary blood returned to the heart, one portion has the same content of gases as venous blood while the other approaches normal arterial blood in its O_2 content but is lower than normal in CO_2. The resultant mixture of blood pumped into the arteries is low in O_2, but near normal, or even below normal in respect to CO_2.

Further progression of edema interferes with the diffusion of gases to an increasing extent. The partial pressure of CO_2 (pCO_2) rises while anoxemia becomes more and more intense, characterized by intense air hunger. The more marked symptoms depend upon the excessive pCO_2 in the arterial blood, but the more serious damage results from the deficiency of O_2. Death of humans from slight exertion can be frequent and is probably a result of the failure of an overworked and asphyxiated heart. The asphyxia of lung edema arising from irritant gases, therefore, is more dangerous than the superficial symptoms may indicate.

3.2.2 Infection as a Sequel to Inflammation.

As mentioned previously, inflammation of the respiratory tract lessens or removes the normal barriers to bacterial invasion. Exposure to

an irritant gas insufficient to affect the lungs directly acts on the higher respiratory structures to cause bronchitis or trachitis. These infections may cause bronchopneumonia as a sequel. In all probability, prolonged inhalation of irritant gases, even in high dilution, may predispose to the development of respiratory infections. A healthy person or animal may carry a dormant infection and the disease may become clinically evident whenever the bodily resistance is lowered sufficiently. Thus, there may be a question in any particular instance whether or not the gassing has undermined the individual's general health to an extent sufficient to allow the active development of pre-existing infections. Henderson and Haggard (60) believe that the infections following exposure to irritant gases constitute a far greater cause of death than does pulmonary edema.

3.2.3 Protective Reflexes.

The protective reflexes play an important role in furnishing a warning of the presence of irritants, particularly NH_3 which attacks the upper respiratory tract and elicits a response in concentrations well below an immediately dangerous level. The reflexes consist in coughing, constriction of the larynx and bronchi, closure of the glottis and inhibition of respiration (60). Coughing is excited by slight irritation of the larynx and tends to expel the stimulating material. But coughing cannot prevent passage of irritant gases - it can only warn of their presence. Higher concentrations stimulate the superior laryngeal nerve which inhibits respiration with the chest in the expiration position. Sneezing, caused by stimulation of the trigeminal nerve in the nasal passages, almost equally disturbs breathing. With severe irritation, reflexes tend to close the glottis and the passages to the lungs by constriction of the adductor and

bronchial muscles respectively. Normally the inhibition of respiration is broken by the increasing excitement of the respiratory center because of the lack of ventilation and the increasing venosity of the blood. Occasionally the spasm of the glottis may persist so long that symptoms of acute asphyxia appear. The constriction of the bronchi may be sufficient to produce areas of atelectasis and emphysema in the lungs (60).

The signs and symptoms arising from inhalation of pollutants thus can be recognized readily. As a rule, coughing, sneezing and dyspnea are reversible immediately upon withdrawl of the subject from the contaminated area. Congestion of the bronchial mucosa, pulmonary edema, shock, and bradycardia are not quickly reversible and may persist even after cessation of inhalation of the pollutant. All these clinical aspects appear to be manifestations of defense mechanisms against the pollutant (10).

3.2.4 Chronic Effects of Exposure to Irritant Gases.

Long-term derangement of health from exposure to irritant gases may arise in two distinct ways (60): 1) As a chronic inflammation following one very severe exposure to the gas; and 2) as a chronic inflammation caused and maintained by continued exposure to low concentrations of the irritant.

A severe irritation of the bronchi and lungs followed by bacterial invasion may result in the infection persisting and causing a protacted period of ill-health. The most characteristic feature of a chronic condition of the bronchi and lungs are bronchitis, fibrosis, and obliteration of a portion of their deeper structure, sometimes with abscess formation.

The distinguishing feature of the second type of chronic inflammation arising from prolonged exposure to sublethal concentrations

is a catarrhal inflammation of the upper respiratory tract. Repeated attacks of respiratory infections are frequent, and the sharp cough present in the beginning of the inflammation usually becomes less marked. The subject then appears to have acquired a partial tolerance to the gas. The appearance of tolerance arises from the fact that the protective reflexes, especially coughing, are less active because of a tenacious mucous covering that partly shields the surface of the upper respiratory tract from the action of the gas. At the same time, the catarrhal exudate does not afford any protection to the deep respiratory structures. Rather, it exposes them more to the action of the gas because of the partial abolition of the respiratory reflexes. In addition, the inflammation diminishes the normal protection against bacterial infection. Hence, repeated exposure may permanently nullify the defense mechanisms so that previously non-lethal concentrations become lethal (10).

The situation of repeated exposure to a pollutant is a complex one because of the question of tolerance. Henderson and Haggard (60) claim that no true tolerance exists toward any irritant gas, but Aviado and Salem (10) have reported the development of tolerance to one irritant (ozone). The complete understanding of chronic effects must await the resolution of problems regarding the mechanisms for the acute effects (10).

3.3 Ammonia.

Ammonia has been classified as a secondary irritant (60) based on the observation that its irritant action may be less marked, particularly in high concentrations, than are its systemic toxic effects resulting from absorption. The gas is irrespirable except in very low concentrations and its violent action upon the upper respiratory tract and eyes gives ample warning of its presence in the air. Table 4 summarizes some physiological responses of humans to NH_3.

TABLE 4: PHYSIOLOGICAL RESPONSE OF ADULT HUMANS TO AMMONIA*.

Effect	Concentration (ppm)
Least detectable odor	5 - 50
Irritating to mucous surfaces (1 hr)	100 - 500
Immediate irritation of eyes, nose, throat	400 - 700
Severe eye irritation, coughing and frothing at mouth, could be fatal	2000 - 3000
Respiratory spasm, rapid asphyxia, may be fatal (40 min)	5000
Rapidly fatal	10,000

* Compiled from references 60,87,101,120.

3.3.1 Absorption, Excretion, Metabolism.

Studies (21) have shown that because NH_3 from the inspired air was absorbed by the mucous surfaces of the noses, mouths, and throats of animals, the tracheae and bronchi were partially protected. As a result, except in cases of exposure to very high concentrations, the lungs may be relatively unaffected. Part of any NH_3 reaching the alveoli is neutralized by the CO_2 normally present, and part may be absorbed unchanged into the circulation (101).

Although the alkaline properties of NH_3 might be expected to upset the bodily pH after prolonged exposure to low concentrations, no data have proven that such is the case. According to Sollman (116), NH_3 in the body is converted rapidly to urea. It is excreted mainly in this form. After exposure, traces of NH_3 have been found in sweat, urine, and exhaled air; at the same time, however, NH_3 is a normal constituent of

blood and urine (101). Little evidence of chronic effects from prolonged daily exposure of animals to concentrations below those causing acute effects has been found (126).

3.3.2 Irritant Action.

Due to the extreme solubility of NH_3 in water, with which it forms ammonium hydroxide, the gas may be absorbed from the inspired air by contact with the first moist tissues in the respiratory tract. As a result the upper respiratory passages tend to bear the brunt of the action (60). The gas tends to destroy the mucous surfaces upon prolonged contact by dissolving or emulsifying keratin, fat, and cholesterol (101).

In addition to its corrosive action on the surface of the respiratory tract, NH_3 can cause temporary blindness (87) and permanent injury to the cornea of the eye (101). A concentration of NH_3 which is intolerable to the eyes and throat of a man may cause no appreciable irritation of dry skin. Patty (101) has noted that atmospheres of 1 per cent (10,000 ppm) NH_3 are mildly irritant to moist skin, while those of 3 per cent or greater cause a stinging sensation and may produce chemical burns with blistering after a few minutes exposure. Concentrations below the amount that causes irritation are not known to have any adverse effect regardless of the length of exposure, but NH_3 may cause sensitization (101).

3.3.3 Systemic Action.

The influence of NH_3 on respiration and the heart is due to reflex action from irritation of the upper respiratory tract. Exposure to high concentrations is followed by intense congestion and swelling of the upper respiratory passages and possibly death from spasm or edema of the larynx. If the concentration is sufficiently high, the lungs may be affected and respiration may be stopped (60). Pulmonary edema has been

cited as the most frequent cause of death in man from NH_3 exposure (101).

3.4 Hydrogen Sulfide.

Hydrogen sulfide is grouped with the inorganic hydric compounds (60) and as such is a general poison. In sublethal concentrations, H_2S is primarily an irritant gas but its systemic action in acute poisoning overshadows the irritant action.

Hydrogen sulfide poisoning has been categorized by the signs, symptoms, and sequelae produced by exposure (91). Generally, 'acute' exposure refers to systemic poisoning that has been of rapid onset where central nervous system effects have predominated, the most dramatic being respiratory paralysis. The term 'sub-acute' has been used to describe cases in which the local irritant effects of H_2S have dominated. There has been no unanimity in recognition of 'chronic H_2S poisoning'. Some readily characterize chronic poisoning as a clinical condition of long-term exposures to low levels of H_2S (67) while others suggest that the condition is actually a series of low-grade acute episodes (2).

In any case, one should understand that both local and systemic injury may result from exposure to H_2S.

3.4.1 Irritant Action.

In low concentrations H_2S has an offensive 'rotten egg' odor and a 'sweetish' odor at higher concentrations. However, with continuous inhalation the olfactory sense fatigues rapidly resulting in no detectable odor.

When H_2S is brought into contact with moist tissue, it combines with the alkali present, and sodium sulfide is formed. Irritation is produced both by the abstraction of the alkali from the cells and by the sulfide which is caustic (60).

Among the subacute and chronic effects of exposure to H_2S, eye irritation or 'gas eye' (130) is the most common (101). A marked irritant action is exerted upon the cornea and conjunctiva, accompanied by pain, inflammation, lacrimation and photophobia. In man, the eyes may itch, smart, and feel as though grains of sand were on the conjunctivae. More severe conditions progress to keratoconjunctivitis (91) with associated clouding of the cornea and vesiculation of the corneal epithelium. Rupture of these vesicles, if followed by corneal ulceration, may heal with scar formation. Permanent impairment of vision is a possible result (91,101). Eye effects have been reported at concentrations of 20 ppm or below (88).

At low concentrations, all of the mucous membranes of the respiratory tract are irritated, causing hoarseness, cough, and nasal secretion (2). Prolonged exposure at concentrations from 50 to 100 ppm may cause rhinitis, pharyngitis, bronchitis and pneumonia (2,83). The most potentially lethal irritant effects of H_2S arise from its ability to penetrate the deepest structures of the lung - the alveoli - producing inflammation (91). The result of prolonged exposure to concentrations between 250 and 600 ppm is likely to cause pulmonary edema or bronchial pneumonia (101).

The local irritant action of alkaline sulfides on the skin results in a softening and subsequent destruction of the stratum corneum (75). In some industrial areas, H_2S has been suggested responsible for allergic eczema (104). Human subjects have noted slight skin irritation in atmospheres containing 2 per cent (20,000 ppm) H_2S (101).

3.4.2 Absorption, Excretion, Metabolism.

The absorption of H_2S into the blood is almost exclusively through the respiratory tract (101). As of yet, however, no work has shown the exact site of entry into the circulation (58).

Henderson and Haggard (60) have stated that the gas is absorbed as alkali sulfide and then hydrolysed in the plasma, liberating free H_2S. In the presence of O_2, Haggard (56) concluded that the free gas was oxidized and destroyed in the plasma. This interpretation conflicts with the results of later work by Evans (49) which showed that the destruction took place, not in the plasma, but in the red cells as a result of the reduction of oxyhemoglobin (HbO_2). Detoxification of H_2S in the blood stream was attributed to the net reaction:

$$HbO_2 + 2H_2S \rightarrow 2H_2O + 2S + Hb.$$

Thus, in the act of causing the deoxygenation of hemoglobin, the sulfide itself necessarily is destroyed. In his proposal, Evans (49) presumed that the sulfur set free by the above reaction would be in the colloidal state and so would remain inside the erythrocyte at least for a time. Its terminal fate may be oxidation to sulfate (41). Having reasoned that inhaled H_2S was carried for a time in the plasma in the form of an equilibrium mixture of ionized sulfide and gaseous H_2S, Evans (49) suggested that H_2S, or rather the sulfhydryl ion (SH^-), penetrated the envelope of the red cells slowly. Therefore, the removal of sulfides from the plasma and their subsequent destruction was relatively slow. Since blood flow time from the lungs to the brain in normal man is only 8 to

10 seconds, very little protection by detoxification in the red blood cells could occur to prevent neural exposure. Adelson and Sunshine (1) have reported that H_2S is promptly oxidized to sulfate and thiosulfate with O_2 from oxyhemoglobin in the red blood cells. Perhaps myoglobin of muscle also assists the blood hemoglobin in destroying sulfides, while another factor involved is the general diffusion of H_2S from plasma to lymph and tissue fluid (49). When free sulfide exists in the circulating blood, an amount of H_2S sufficient to be detected by odor can be excreted in the exhaled breath (101). A greater fraction, however, is eliminated through the kidneys as sulfate or sulfide in the urine (49,101). Obviously, knowledge is incomplete as to the means by which detoxification of H_2S is effected in the body.

Percutaneous absorption of gaseous H_2S is probably not a significant source of systemic poisoning. This is evidenced by the fact that respiratory devices covering only the face and head will permit work in H_2S atmospheres which would otherwise be immediately fatal (101,130). Nevertheless, some controversy persists. Exposure of pure H_2S gas to the intact skin of the guinea pig has proven fatal in about 45 min (125); but subsequent experiments have failed to confirm these results (130). Petrun (103) has captured H_2S in the expired air of rabbits subjected for 2 hours to 1400 ppm at skin level while breathing normal air. Laug and Draize (75) have shown that poisoning from H_2S in combination with NH_3 gas occurred more rapidly than from H_2S alone when these compounds were in contact with moist skin. Abrasions of the skin facilitated the absorption of both H_2S and ammonium hydrogen sulfide (NH_4HS) gases. These experiments did not show why NH_4HS penetrated the skin more rapidly than H_2S. Laug and Draize (75) postulated that the irritating properties of the ammonium

radical may account in part for the erythema and congestion of the skin. This could favor absorption because of the increased blood supply; on the other hand, the properties of molecular NH_4HS may be such as to favor its penetration over that of molecular H_2S (75).

3.4.3 Systemic Action.

Although the degree, rate and site of metabolism are still nebulous, the literature is in general agreement that the systemic effects of H_2S occur only when free, unoxidized H_2S gas is present in the blood stream (1,60,91,101). The earlier proposal that the physiological effects were induced by removal of O_2 from the oxyhemoglobin in the blood and consequent asphyxia no longer appears to warrant consideration. Lack of O_2 could not be the cause because even a rapidly administered lethal dose of H_2S can deoxygenate only about 1 per cent of the circulating blood (49).

The problem of the action of H_2S on the blood has been obscured by the work carried out on the formation of sulfhemoglobin. Haggard (60) asserted that in vivo, H_2S does not combine irreversibly with oxyhemoglobin to disturb the oxygen-carrying capacity of hemoglobin; the gas, however, does combine with methemoglobin (49,60). This substance is normally present in the blood in only small amounts, but sulfmethemoglobin appears in the blood of cadavers resulting from post-mortem changes (60). Notwithstanding these assertions, Laug and Draize (75) reported that the blood of animals whose skin was exposed to NH_4HS carried from 10 to 20% saturation with sulfhemoglobin; the animals also exhibited signs of O_2 deficiency. These authors noted that the detection of sulfhemoglobin may be dependent upon some other mechanism such as methemoglobin formation. In any case, Laug and Draize (75) reasoned that a 20% saturation of the blood with sulfhemoglobin scarcely could be the sole factor involved with

the slow deaths, and certainly indications were that sulfhemoglobin itself is non-toxic. Interestingly, in immediate death resulting from intravenous injection of NH_4HS, no sulfhemoglobin was detected even though comparable amounts to blood in vitro produced detectable amounts of sulfhemoglobin (75). Evans (49) conceded that, in vitro, in addition to the main reaction - the simple reduction of oxyhemoglobin - part of the hemoglobin was converted into an unknown degradation product by the action of sulfide. This greenish derivative of hemoglobin together with oxidation products of H_2S was irreversible, as indicated by the fact that the initial O_2 capacity of the blood was not restored upon reoxygenation of blood after sulfide reduction (49). Evans (49) concluded that in vivo the extent of formation of such a hematin-like fraction is a matter for speculation, but apparently the reduction of oxyhemoglobin is completely and rapidly reversible in vivo. To cause system intoxication, then, gas must be absorbed at a rate faster than it can be eliminated or detoxified into pharmacologically inert compounds, such as thiosulfate and sulfate. Relatively massive doses of H_2S are required to overwhelm the protective activity in the body, primarily due to the fact that H_2S is rapidly oxidized and detoxified in the blood (49,60,101).

The most conspicuous systemic action of H_2S is occasioned by free gas in the blood acting upon the nervous system (49,60). However, suggestions also have been made that myocardial infarction, elevation of blood non-protein nitrogen levels, and appearance in the urine of red blood cells and hyaline casts may be associated with H_2S intoxication (72).

In small amounts, H_2S depresses the nerve centers, in larger amounts it stimulates, and in very large amounts it paralyzes (60). Low concentrations (< 150 ppm) may cause headache, fatigue, irritability,

insomnia, and gastrointestinal disturbances. In somewhat higher concentrations (500 to 600 ppm), H_2S acts as a nervous system stimulant causing excitement and dizziness (91). The pupils are constricted, respiration is increased, and blood pressure is raised (49).

Death in systemic poisoning results from respiratory failure with consequent asphyxia (60,75,91). This respiratory failure may be brought about in one of two ways, depending upon the concentration of the gas inhaled (60). Moderately high concentrations (500 to 600 ppm) of H_2S stimulate the respiratory center, and hypernea results. Excessive CO_2 is blown-off by this overbreathing with a concomitant decrease in alveolar (and arterial) pCO_2. Apnea then leads to asphyxia (91). The stimulatory effects on respiration may be due to either a direct action on the respiratory center in the brain, or it may be mediated reflexly in some way, as via the vascular chemoceptors (49).

In very high concentrations (> 600 ppm), H_2S exerts a direct paralyzing action on the respiratory center, producing respiratory failure and asphyxia (60,75). The inhalation of H_2S in a high concentration may cause unconsciousness after a single breath, and death is rapid (91). The mechanics of this respiratory paralysis formerly were thought to involve a chemical reaction with the respiratory enzymes or with the hemoglobin or both, but are now believed to be due to reflexes resulting from irritation of the carotid sinus (101). In less severe poisoning, convulsions and dyspnea are marked symptoms (60).

Because of the rapid oxidation of H_2S in the blood, the symptoms of acute poisoning pass off when inhalation ceases (60). Also, low concentrations (e.g. 20 ppm) can be breathed for long periods of time without apparent harm (49). Both of these observations indicate that H_2S

is to a high degree a non-cumulative poison; thus, in non-fatal poisoning, systemic sequelae are uncommon (2,83). However, when they do develop, they can be explained generally as resulting from damage of the central nervous system produced during the period of collapse and anoxia attendant with H_2S asphyxia (91). Ahlborg (2) pointed out that most patients experiencing sequelae after poisoning without unconsciousness had undergone repeated episodes of intoxication. Further, his reports suggest that the sequelae resulting from mild, repeated exposures are transient in nature. In chronic poisoning the main symptoms are those of irritation, particularly of the eyes and, to a lesser degree, of the respiratory tract. In some cases a mild degree of malaise gives evidence to the depressing action of the gas (60). Tolerance to H_2S does not develop; in fact, hypersusceptibility to the effects of the gas have been noted following exposure (2). Some of the more prominent responses of humans exposed to H_2S are summarized in Table 5.

TABLE 5: PHYSIOLOGICAL RESPONSE OF ADULT HUMANS TO HYDROGEN SULFIDE*.

Effect	Concentration (ppm)
Least detectable odor	0.01 - 0.7
Offensive odor	3 - 5
Eye irritation	10
Irritation to mucous membranes and lungs	20
Irritation of eyes and respiratory tract (1 hr)	50 - 100
Olfactory-nerve paralysis, fatal in 8-48 hr	150
Headaches, dizziness (1 hr), nervous system depression	200
Nausea, excitement, insomnia, unconsciousness, possible death (30 min)	500 - 600
Rapidly fatal	700 - 2000

* Compiled from references 58,60,91,93,101,120.

4. Responses of Farm Animals to Ammonia and Hydrogen Sulfide.

The clinical signs and symptoms from any toxic gas cannot be categorized rigidly because the effects depend so much upon the duration and intensity of exposure, as well as varying with species, age, sex, size and body weight. For these reasons, the effects of manure gases on the response and performance of cattle may be better appreciated by surveying the literature for comparative effects in other farm animals.

Due to the combination of gases arising in animal accomodation, it is difficult to isolate any one gas as the cause of harmful effects; indeed, it is possible that the toxicity increases with mixture (14,58). However, the simple asphyxiants, namely CO_2 and CH_4, are probably not a critical problem. Firstly, apparently the concentrations of CO_2 experienced in normally ventilated buildings exert no injurious physiological effects (14). Furthermore, neither CO_2 nor CH_4 have been measured in cattle buildings at concentrations that are considered dangerous to humans (55,97). Secondly, the suggestion even has been made that calf health and performance are considerably better at CO_2 levels of 0.5% (5000 ppm) than at a level of 0.1% of CO_2 (14). Interestingly, the CO_2 TLV for human exposure is 5000 ppm (5). Thirdly, there is abundant knowledge on the response to various concentrations of asphyxiants, and their effects can be applied generally to both man and animals (60).

On the other hand, H_2S alone (16,17,64,68,99) and in combination with NH_3 (55,67) has been implicated as the principal offender in cattle poisonings attributed to manure gases. That H_2S and NH_3 are capable of killing animals is no longer subject to debate. Both lethal and potentially harmful sublethal levels have been reported in cattle facilities (3,55,68,97). To obtain reliable information from man

or other species regarding either the local irritant effects, or the sub-acute systemic effects of H_2S and NH_3 is virtually impossible (60). But comparative effects in different species are valuable as a guide, and provide a background on the generalities of these pollutants. With regard to the different types of livestock, needed design data are most nearly adequate for poultry and swine housing and least adequate for beef and dairy calves (59). In view of this fact, much of the existing information cited on the response of poultry and swine to NH_3 and H_2S will be studied briefly but intensively. In addition, the reports of cattle poisonings will be reviewed. For the sake of simplicity and clarity, the survey has been subdivided on the basis of pollutant and species of animal.

4.1 Exposure to Ammonia.

4.1.1 Poultry.

Since poultry confinement preceded livestock confinement in most countries, poultrymen encountered air-factor constraints on performance before livestock producers. Accordingly, a relative preponderance of literature referred to exposure of poultry to various levels of atmospheric NH_3.

Clinical Symptoms and Findings.

Bullis et al (28) first described an ocular disorder under the name of keratoconjunctivitis; the condition appeared to be associated with atmospheric NH_3 in poultry houses. Attempts to demonstrate an infectious cause were unsuccessful (129). In another early case (11), keratoconjunctivitis developed by the 16th week of age in 25 per cent of the chickens exposed to a highly concentrated NH_3 atmosphere produced from decomposition of accumulated droppings; in most instances

the removal of affected birds to clean environments resulted in rapid recovery. Following these initial reports, keratoconjunctivitis has been reproduced experimentally by exposing chickens to NH_3 fumes (7,31,50,53, 110,129).

The condition may affect either one or both eyes and is characterized by a thickening and opacity of the cornea. In milder cases, the cornea is roughened and shows a slight opacity while, in more severe cases, the cornea is thickened and shows deep erosions on its surface. Affected birds tend to keep their eyes closed and may rub them on the wing. Frequently, the conjunctiva is also involved; it is congested and later becomes edematous resulting in slight eversion of the eyelids. Wright and Frank (129) noticed lesions in the posterior half of the eye at first, later the entire cornea became involved. Histopathological study showed that in some places erosions penetrated the entire thickness of the corneal epithelium. In the eroded region, Bowman's membrane* was absent and there was a cellular reaction consisting of lymphocytes, histocytes, fibroblasts, and eosinophils in the anterior layers of the substantia propria**. In experiments at 100 ppm NH_3, Charles and Payne (34) noted symptoms of keratoconjunctivitis in the sixth week, thereafter about 10 per cent of the fowl developed severe ulcerations. Other chickens exposed to 200 ppm NH_3 showed rubbing and lacrimation of the eyes during the first few days, but all eye irritation disappeared by 17 days of exposure (7). After 8 days exposure to 1000 ppm NH_3, corneal opacities and surface erosions appeared; by 14 days nearly all birds exhibited bilateral opacities of

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* The membrane separating the corneal epithelium from the substantia propria.

** The central, transparent, lamellated layer of dense connective tissue in the cornea.

varying severity (7). In another case, only 60 ppm NH_3 was needed to produce keratoconjunctivitis (79). Gaspar et al (53) reported the characteristic condition and even unilateral or bilateral blindness in chickens exposed to approximately 45 ppm NH_3 for a period of 12 weeks. Obviously the severity and incidence of keratoconjunctivitis varies, but tends to increase with NH_3 concentration and exposure time.

Some experiments, (33) have indicated that one fundamental cause of the effects of NH_3 on production parameters appears to be a reduction in respiratory turnover. Exposure of hens to 100 ppm NH_3 caused a reduction in the respiration rate of between 7 and 24 per cent even after the hens had become accustomed to the polluted atmosphere; and a reduction in CO_2 production and respiratory depth. After 15 minutes at 75 ppm, a slight rise in blood pH occurred (6.447$\pm$0.470 prior to exposure, and 6.563$\pm$0.072 after exposure). Charles and Payne (33) speculated that ammoniated alveolar air may give rise to NH_3 by-products in the blood which alter its pH. Thus, in a manner analogous with the regulation of respiration rate by CO_2, respiratory activity might be reduced.

Chickens and turkeys showed no histopathological changes until the sixth week of exposure to 20 ppm NH_3 (7). At that time the most notable changes in the respiratory tract were pulmonary edema, congestion, dilation of veins and capillaries and hemorrhage. Ciliary loss and increased goblet cells also have been detected in both the nasal and tracheal epithelium of birds exposed for 6 days to 30 ppm NH_3, 0.39 mg/cu ft dust, and 5000 ppm CO_2 (8). Apparently damage to the respiratory

tract from low concentrations of NH_3 would likely go undetected on gross or histopathological examination, but this damage became apparent when chickens were subjected to a respiratory infection (7).

The hemoglobin content of avian blood ultimately was suppressed significantly upon exposure of month-old chicks to about 45 ppm NH_3 for 12 weeks; an initial significant elevation in blood hemoglobin occurred after four weeks of exposure (53). Evidently the rate of red blood cell formation was stimulated by the gradually increasing levels of NH_3, but continuous concentrations of 45 ppm interferred in some way with the normal utilization of iron in hemoglobin formation. Other symptoms include head jerking at the 75 ppm level (79) and huddling together of affected birds (123).

Production.

Apparently hens' comfort needed for high egg production becomes affected then NH_3 exceeds 20 ppm (48). Layers exposed at 18°C for 10 weeks to 100 ppm did not lay as well as those exposed to 50 ppm or those which were unexposed, and withdrawal of NH_3 did not permit egg production to return to normal (48). Consistent with these results, Charles and Payne (34) found egg production from hens exposed for 10 weeks at 19°C to 105 ppm NH_3 was significantly less than that of control hens or those housed in 52 ppm NH_3. Production failed to improve during a 12-week recovery period in an atmosphere free of NH_3. A repeat trial indicated that the effect of the same concentration (102 ppm) was more marked at 28°C; egg production fell significantly below the other two treatments (0 and 50 ppm) within seven weeks. Exposure of pullets to 53 and 78 ppm NH_3 from 11 through 46 weeks of age significantly increased age at sexual maturity by one and two weeks respectively (33).

Apparently high NH_3 concentrations exert no adverse effects on the dressing quality of broilers (79). Levels up to 75 ppm NH_3 did not retard growth but induced an unhealthy appearance in chicks (79). A level of 78 ppm NH_3 significantly reduced feed consumption of pullets from 15 to 30 weeks of age and liveweight gains from 15 to 22 weeks of age while 106 ppm NH_3 administered to cockerels from 28 to 47 days of age caused a reduction in food consumption of 14.5 per cent (33). Other studies (34) showed that feed intake and weight gain was deterred by 105 ppm NH_3 for 10 weeks. No significant differences in weight gains were noticed between chickens and turkeys exposed to 20 ppm and control birds over a 6-week trial (7). The salient feature throughout the trials reported by Charles and Payne (33,34) was the reduction of appetite when poultry were housed in atmospheres polluted with NH_3. This was probably in part due to the reduction in respiration, as discussed by the authors: "Since respiratory evaporation contributes to heat losses of the fowl, it appears that ammonia mediated reduction in respiration rate must be associated with a reduction in heat losses. Hens are homeotherms and, as Blaxter (20) has pointed out, any homeotherm faced with an involuntary reduction in heat loss must eventually reduce metabolic heat production. Thus it is logical to assume that the reductions in appetite caused by ammonia are at least in part due to reduced energy requirements caused by lowered body heat loss".

Numerous effects have been attributed to the reduced food consumption of birds in ammoniated air, resulting in deficiency of nutrients. Thus, body weight has decreased and production tended to decline. Post-mortem examination of birds which died during exposure to 102 ppm NH_3 revealed symptoms of calcium deficiency, such as weak ribs and long bones; the effects were referred to the much reduced food consumption during the

treatment (34). Charles and Payne (34) have stated that NH_3 toxicity is, in effect, a shortage of protein, vitamins, minerals and essential amino acids, and have alleviated the detrimental effects of NH_3 in hens by providing a ration highly supplemented with these nutrients. To describe and explain this debilitating consequence of NH_3, these authors have suggested the term 'environmentally induced nutritional stress'.

Resistance to Disease.

In poultry houses NH_3 concentrations may well reach 50 to 100 ppm when ventilation rates are low (9). Such levels for prolonged periods have been reported to predispose chickens to respiratory diseases with the added risk of secondary infections (14,79,123). When chickens were exposed to an aerosol of Newcastle disease virus, the infection rate was significantly increased by a previous 72-hour exposure to 20 ppm NH_3 or by a 48-hour exposure to 50 ppm NH_3 (7). Ernst (48) reported identical results from exposure to 20 ppm NH_3 for three days; however, a different strain of Newcastle disease virus did not increase infection rate in hens exposed to 30 ppm for six days.

4.1.2 Swine.

Clinical Symptoms and Findings.

Decreased rates of gain have been reported in pigs weighing about 150 lb (68 kg) when the animals were reared in confinement buildings with underfloor wastes ponded for a month or longer (40). Others (14,96) have reported that concentrations of 100 to 200 ppm NH_3 may result in anorexia. Subsequently, reduced average daily gains have occurred in market-weight pigs exposed to 100 ppm NH_3 for a 5-week period (118). The decrease in weight gain was due to a decrease in feed intake and not to a decrease in feed efficiency. Doig and Willoughby (42) were unable to confirm these

findings in weanling pigs exposed for a period of up to six weeks to 100 ppm NH_3 alone; but a combination of NH_3 and corn starch dust (6 mg/cu ft) caused pigs to stand with their heads lowered at times when they normally would be sleeping and reduced appetites when NH_3 concentrations exceeded 125 ppm. However, in these experiments feed intake was not measured and limited feeding contributed to a considerable degree of competition between pigs at feeding time. As viewed by the authors (42), this competition may have masked a decrease in feed intake that might have been apparent if feed had been provided for a longer period.

The combined effect of aerial dust and NH_3 was further demonstrated by trials (38) in which NH_3 alone at 50 or 75 ppm had little effect on daily gains of pigs; only when hog-house dust was applied at a very high level (300 mg/m^3) with 50 ppm NH_3 were daily gains reduced. The effect of dust and NH_3 were additive; aerial dust apparently did not potentiate the assault of NH_3 on the pig.

Signs of conjunctival irritation, including photophobia and excessive lacrimation were observed in a few pigs during the first week of exposure to 100 ppm NH_3; thereafter the pigs appeared to acclimatize and irritation was no longer apparent (42). Increased concentrations of more than 150 ppm NH_3 caused pronounced signs of conjunctival irritation in all the pigs. Concentrations of 100 to 200 ppm NH_3 have caused swine to sneeze and to salivate (96). Nasal, lacrimal and mouth secretions have been more excessive in pigs at 100 and 150 ppm than at 50 ppm NH_3, with symptoms in all animals appearing to lessen after one or two weeks exposure (118). When subjected to 280 ppm NH_3 (118), a 66 lb (30 kg) gilt frothed at the mouth at first; after three hours she exhibited excessive secretions about the mouth and nose, a short and irregular respiratory

pattern, and occasional sneezing and shaking of the head. By 36 hours, convulsions occurred and breathing was extremely short and irregular. The pig continued to have convulsions for three hours following stoppage of the gas. A further seven hours passed before the animal appeared normal, except for intermittent sneezing and head shaking. Increased coughing has (118), and has not (42) been noted in exposed pigs. Apparently, higher NH_3 concentrations (100 and 150 ppm) may increase the frequency of coughing three times as much as lower levels (50 and 10 ppm) (118).

Examination of the respiratory tract of pigs averaging 138 lb (62.5 kg) and subjected to four levels of NH_3 (approximately 10,50, 100 and 150 ppm) for five weeks revealed no significant gross or microscopic differences related to contamination level (118). In accord, Curtis et al (38) reported no evidence of consistent gross or microscopic structural alterations of the respiratory tract in swine resulting from treatments of 50 or 75 ppm NH_3 for up to 109 days. Histopathologic changes following exposure of weanling pigs to 100 ppm NH_3 were limited to the upper respiratory tract, and consisted of an increased thickness of the tracheal epithelium and a corresponding decrease in goblet cell* numbers after two weeks of gassing (42). In the same experiment, during a combined 100 ppm NH_3 and ground corn dust (0.3 mg/cu ft) exposure, there was also a noticeable decrease in goblet cell numbers after the fourth week, leading the researchers to believe that decreased goblet cell numbers are the first response to respiratory tract irritation. However, as pointed out (42), indications are that a number of factors, including the nature and

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* One of the unicellular mucous glands found in the epithelium of the mucous membranes of the respiratory passages.

concentration of the irritant, the duration of exposure, and perhaps the species of animal, influence goblet cell response. The lack of changes in the bronchi, bronchioles, and alveoli of NH_3-exposed pigs is consistent with the proposal that a large portion of NH_3 is removed in the upper respiratory tract (42).

The fact that high levels of NH_3 interferred with the normal utilization of iron in hemoglobin formation in chicks was interpreted (53) to suggest that high levels of atmospheric NH_3 in farrowing houses may be a predisposing factor in the occurrence of iron-deficiency anemia in baby pigs. There were no significant differences in the packed cell volumes, total white blood cell counts, differential leukocyte percentages, or total serum lactic dehydrogenase activities when results from exposed (100 ppm NH_3) and control pigs were compared (42).

Ammonia Toxicosis via Injection.

Experiments (18) with young pigs indicated that toxicosis caused by intravenous infusion of various NH_3 salts was primarily due to the ammonium ion. Before death the symptoms were rapid respirations, becoming irregular and increased in depth, excessive salivation, and clonic-tonic convulsions; no significant gross or microscopic lesions were observed. Reduction of proteins in the diet increased the blood NH_3 values when the pigs were injected intraperitoneally with ammonium acetate.

Effects of Surgical Alterations of the Liver on Ammonia Metabolism.

Clinical signs of icterus appeared within 12 hours in pigs with surgically produced biliary occlusion. Bilirubin levels generally increased up to the fifth day, then receded. In a few pigs, levels of the blood enzyme serum glutamic-oxalacetic transaminase increased markedly 24 to 48 hours prior to death. These values correlated with histopathologic

findings of extensive necrosis (18).

General Health and Disease.

In swine, noxious gases have been implicated as contributing factors in the pathogenesis of enzootic pneumonia (70). Baxter (14) has mentioned that high concentrations of NH_3 in piggeries can influence the occurrence of lung disease in piglets. Field studies supporting these contentions have reported an increase in both the incidence and severity of pneumonia in swine housed in barns with high NH_3 and dust levels (42). Chronic coughing and reduced growth rates also have been reported in swine confined in barns with high odor levels (6).

Ammonia gas can prove menacing by an indirect phenomenon. When present in the atmosphere, NH_3 may condense on the walls of a piggery, subsequently be oxidized to nitrite or nitrate, and accumulate in the pens. Access to these compounds may cause nitrite or nitrate poisoning in pigs (81).

Similar changes found in the tracheal epithelium of pigs after two weeks exposure to 100 ppm NH_3 have been reported in the turbinate epithelium of swine with early atrophic rhinitis* (42). Doig and Willoughby (42) thought that although the structural damage to the upper respiratory epithelium of pigs exposed to 100 ppm NH_3 was rather slight, the associated functional impairment may be quite severe; furthermore, these factors may explain in part the apparent increase in the incidence and severity of enzootic pneumonia in swine housed under poor environmental conditions. However, dissenting evidence was produced (38) when levels of NH_3 (50 or 100 ppm), H_2S (2 or 8.5 ppm), and hog-house dust (10 or 300 mg/m^3) alone

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* Inflammation, followed by degeneration of the nasal mucous membrane,

and in various combinations had little effect on growth performance of otherwise healthy pigs. Although the authors (38) conceded that lung disease still may be related to the stress caused by such irritants as NH_3, if lung disease were exacerbated by air pollutants, they expected pig performance to decline in turn as an indirect effect of the pollutants.

Cultures of Corynebacterium and Pasteurella were isolated from the ethmoid turbinates of two pigs exposed to 150 ppm and from one pig maintained at 100 ppm whereas there was no evidence of these bacteria from other pigs subjected to 10 or 50 ppm NH_3 (118). In contrast, other pigs exposed to 100 ppm did not differ from controls in either type or frequency of bacteria from tracheal swabs (42).

4.1.3 Cattle.

Toxicity in cattle from NH_3 gas under field conditions usually is associated with other gases and as such is discussed with exposure of cattle to H_2S.

Ammonia Toxicosis via Injection.

Acute NH_3 toxicity in cattle was produced by adding a water solution of urea directly to the rumen through a permanent fistula (25 g urea/100 lb body weight, or 10 g urea/100 sq in. of estimated rumen surface area) (108). Average blood NH_3 values, with corresponding physical observations were: 1.5 µg NH_4^+ - N/ml, normal; 9.3 µg NH_4^+ - N/ml, muscular twitches; 13.0 µg NH_4^+ - N/ml, recumbency; 14.8 µg NH_4^+ - N/ml, tetanic convulsions. An initial rise in blood pH was reversed, resulting in a continued decline. The packed cell volume of the blood (PCV) decreased initially, then increased markedly by the time of tetanic convulsions. The data for plasma and red cell solids indicated that a loss of water was concurrent with an increased PCV, a decreased blood pH and an elevation of

blood NH_3 levels. The concentrations of plasma Na, K, Ca, and Mg did not suggest any physiologically significant changes; however, red cell Na concentration was lowered and red cell K concentration was increased. A loss of red cell Na and an increase of plasma K was indicated.

Defecation was frequent prior to convulsions, but urination was infrequent. Respiratory movements were initially slow and shallow, but increased to vital capacity as muscular activity and blood NH_3 increased. Respiratory compensation correlated with changes in blood pH; exchange was at a minimum at the height of metabolic alkalosis and at a maximum during metabolic acidosis. Death was attributed to a metabolic acidosis due to the high levels of ammonium ions and their influence on normal metabolic reactions (108).

4.2 Exposure to Hydrogen Sulfide.

4.2.1 Poultry.

Although there had been no reports of fatalities among poultry attributable to toxic gases, McAllister and McQuitty (80) found that agitated poultry slurry can release highly toxic concentrations of H_2S. Measurements near the surface of slurry in tanks after agitation varied from only 2 ppm to in excess of 1000 ppm. The egg production of hens housed for two months in an atmosphere containing 80 to 100 ppm H_2S, 52 to 80 ppm NH_3 and 5000 to 9000 ppm CO_2 has been reported to have fallen by 9 per cent (102).

4.2.2 Swine.

Heavy casualties among pigs have occurred in several instances while slurry tanks above slatted-floor pens were being emptied (65,76,80, 81,84,90,93). In most cases, the toxic agent was believed to be H_2S. McAllister and McQuitty (80) found that while CO_2, CH_4 and NH_3 did not

reach toxic levels in the atmosphere of hog barns following agitation of sub-floor excreta, H_2S reached toxic levels after two to five minutes agitation, and even manual stirring could produce dangerous levels. Concentrations in excess of 1000 ppm H_2S have been reported during agitation of slurry (80). Static liquid manure has released H_2S in measurable quantities only if it originated from pigs (115).

Clinical Symptoms and Findings.

Experimental exposures of 30 to 35 lb (14 to 16 kg) swine to graded levels of H_2S from 50 to 1200 ppm for periods up to four hours have been summarized by O'Donoghue (100). Immediate spasm and death resulted from one sudden exposure to a high concentration. Symptoms seen during other exposures, in order of appearance with rising gas concentrations, were: 50 to 100 ppm, discomfort, slight eye irritation, salivation with periodic swallowing, distress; 500 to 900 ppm, semicomatose state. There followed intermittent muscular spasms, shallow breathing, and a developing cyanosis. Dyspnea was not a prominent feature. Death at concentrations at or above 1000 ppm was sudden and accompanied by tetanic convulsion. No chronic effects were observed in animals surviving exposures as great as 1000 ppm. Furthermore, recovery was rapid. Post-mortems revealed no significant pathology in immediate deaths; in others, there was a superficial cyanosis most marked on dependent portions, but absent in areas where the body was in contact with the floor. Minor hemorrhages were observed in the lungs, but hypostatic congestion of the ventral lung was the most striking feature. In a rabbit dying two hours after a sudden exposure to 1000 ppm, severe pulmonary hemorrhage with epistaxis and distention of the right ventricle were prominent features. In no case was there a discernable odor of H_2S to the carcass, and no discoloration of viscera was observed. The author's

opinion was that a confirmed diagnosis would have to be based on a known exposure; pathology and toxicological examination of tissues or organs will not supply confirmatory evidence. Furthermore, O'Donoghue's impression was that the toxic effects of H_2S were related more to the concentration of the gas than to the length of exposure time, and that sudden exposure may reduce the minimum lethal concentration.

A report (93) of manure gas poisoning of 150 to 200 lb (68 to 91 kg) feeder pigs described affected animals as lying on the floor or staggering and breathing with difficulty within five minutes after agitation of excreta began. Chemical analysis of the slurry found H_2S and NH_3 and carbon monoxide present but, from the rapidity of deaths, H_2S was concluded as the principal toxic agent. In this case, recovery of survivors was gradual, rather than rapid as observed by O'Donoghue (100). Hours after exposure, the pigs showed listlessness, a staggering gait and cyanosis of the lower parts of the abdomen. Rectal temperatures were normal and a blood sample demonstrated no abnormal blood cell types. The significance of a low hemoglobin (2 g/100 ml) and a total white cell count (5500/cmm) in the blood sample was unknown.

Necropsy of dead pigs revealed extreme edema of the lungs with congestion and emphysema. No lesions were found in the spleen, liver, kidney, cerebrum and cerebellum (93). Examination of various tissues from pigs exposed to 8.5 ppm or 2 ppm H_2S for at least 17 days produced no evidence of physiological alterations (38).

General Health and Disease.

Constant exposure to low levels of H_2S has been suspected of being responsible for illness and reduced performance of swine (14,40,90,100). There is evidence that when disease agents of the respiratory tract are

present, air pollutants increase the severity of respiratory disease in swine, with a 7 to 15 per cent reduction in average daily gain having been reported in studies (122).

Twiehaus (122) has pondered why some swine that are confined over pits sometimes vomit. Hydrogen sulfide, either alone at 8.5 ppm or at 2 ppm in combination with 50 ppm NH_3 had a slight deleterious effect on the rate of gain and on feed efficiency of experimental pigs (38). Daily gain was affected to a greater degree by the H_2S alone, but neither treatment was statistically significant compared to controls.

4.2.3 Cattle.

Acute Poisonings.

Acute poisonings usually have occurred in connection with agitation of liquid manure which has been stored for a long time. The development of poisoning is very rapid. Hudek (68) reported the death of two feeder steers within five minutes after pit agitation started while Ober (99) cited the death of eight cows due to H_2S which occurred within 30 minutes, even though the doors and windows were opened before stirring began. A case of asphyxiation of cows, caused by a combination of dung agitation and bad ventilation, was described by Haartsen (55). He concluded that the cows reacted clearly: watering eyes, coughing and slobbering were symptomatic of NH_3 effects (700 ppm was measured near the cows); the cows must have been intoxicated as well (600 ppm H_2S was measured near the cows). Blaser and Studer, cited by Hogsved and Holtenius (67), reported on the pathological-anatomical symptoms of acute manure gas (H_2S) poisoning in domestic animals. Symptoms were characterized by a general tendency to hemorrhages and degenerative processes in the visceral organs. Other observations may be convulsions and dyspnea, but most notable is hemorrhage and extravasation. Post-mortems have revealed

severe lung edema, extensive hemorrhaging in muscles and viscera, edema in the brain, nonpurulent encephalitis, and an offensive smell of H_2S from the carcass (67).

Exposure to Hydrogen Sulfide via Injection or Ingestion.

Hydrogen sulfide gas given to cows and sheep via rectum or stomach (43) decreased the CO_2 content of the blood, depressed the respiratory center, and caused death. Carbon dioxide given intravenously increased the tolerance for H_2S and artificial respiration prolonged life, but neither prevented death of H_2S-intoxicated animals.

Coghlin (36) reported on the poisoning and death of a considerable number of feeder cattle believed due to the formation and absorption of H_2S gas following the consumption of powdered sulfur. Affected steers struggled in convulsions or lay in a coma, and the odor of H_2S was detected easily on their breath. The symptoms in earlier stages included twitching of muscles, especially those of the jaws, eyelids and ears; evidence of pain by lying down and getting up, switching and uneasiness; a staggery gait; and development of severe scouring. Advanced cases were unable to rise, acted blind, and struggled a great deal with grunting and fast, labored breathing. Finally, the animals became comatose and died.

On the whole, provision of fresh air, covering for warmth, tubing with chemical antidotes and other immediate treatments did not seem to check the progress of the symptoms. Following the initial losses, later cases appeared to have lost motion in the rumen and intestines, and there was a complete loss of appetite. Nearly all cattle which showed sickness ultimately died.

A post-mortem examination revealed that the lungs were very dark in color, congested and edematous. The liver was light in color, the

spleen was normal in color, and some of the muscles over the loins and back were almost black in color. The stomachs showed only slight inflammation but the intestines were acutely inflammed.

Chronic (Sub-acute) Poisonings.

Hofmann (63) appears to have been the first to show that cowsheds with liquid manure became contaminated with considerable amounts of H_2S during agitation of the slurry. He mentioned that manure gases could cause abortion in cows and indicated that these gases were connected to cases of respiratory diseases. Further data regarding chronic manure gas poisoning in cattle have been presented subsequently. For the most part, reports have been from Sweden (16,64,97). Hogsved and Holtenius (67) have reviewed the chronic conditions. Development of the illness can be very drawn out and may take a long time for visible symptoms to develop. Dairy cows lose flesh, decrease milk yield, and exhibit inappetence and dull, rough coats. The possibility that manure gases might cause teat injuries also was noticed (65). The more striking changes, however, are in the hoofs and in the general tendency to hemorrhage. Often sore, tender feet and lameness are caused by an excessive softening of the horn in the hoofs which is followed by deep infections. Hemorrhages are common in the horn of the hoofs, and sometimes cracks in the back of the hoof sole penetrate the pododerm, providing an opening for dermatitis and osteitis. Cows may stand with crossed or bent legs, arched back, and develop bedsores and other pressure damage where they rest on stall frames to relieve weight on the feet (64). Subcutaneous hematoma, often larger than a man's head, have appeared, while hemorrhages in muscles and visceral organs have been found at post-mortems. Very extensive hemorrhaging also has occurred with calving. Even in cows without manifest illness, the blood coagulation time was prolonged (16,109). Sallvik (109) noted that the coagulation was

normalized within a few hours after stopping agitation of manure which had produced about 1 ppm H_2S in the animal area. One explanation offered was that the cows might have absorbed the gases through their skin.

Blood examinations have shown lowered albumin/globulin ratios and anemia. Dyspnea and tachycardia have been other symptoms; extreme cases had respiratory rates of 70 to 80 per minute and pulse rates over 150 per minute. Intensified pulsation in the digital arteries also may be common (67). The respiration rates of cows generally increased immediately after dunging out and remained high for one to two hours after stopping agitation. These effects were observed when the H_2S-concentration in the respiration area was about 1 ppm (109).

Hogsved and Holtenius (67) have suggested that the chronic manure gas poisoning could be chronic ammonium hydrogen sulfide (NH_4SH) poisoning for basically two reasons. Firstly, experiments (75) have shown that simultaneous exposure to NH_3 and H_2S results in a more pronounced poisoning effect than with only H_2S. Hogsved and Holtenius (67) noted that the effect of NH_3 and H_2S has been purported to be the same as that of NH_4SH and, hence, NH_4SH possibly may be formed in the animal after inhaling NH_3 and H_2S in manure gas. Secondly, as emphasized by these authors, a prominent characteristic of NH_4SH is its obvious ability to soften a horny substance. In conclusion, Hogsved and Holtenius (67) conceded that naturally other gas components also may be contributing factors.

Information on the effects of long-term exposure of cattle to low levels of H_2S under controlled conditions is very sparse. What few results that are available do not always compare with reports obtained in the field, as verified by Hays (58) who studied the effects of H_2S on ruminants-dairy cows and goats.

In goats, respiration frequency was not significantly different from control periods for exposures of 10 and 50 ppm H_2S, while frequency decreased significantly in goats exposed to 100 ppm H_2S. The effect of exposure to 10,50, or 100 ppm H_2S on cardiovascular function, measured by heart rate and blood pressure, was inconclusive with goats. Likewise, exposure of dairy cows to 20 ppm H_2S for three weeks did not affect cardiovascular response as indexed by heart rate. The rectal temperature of goats exposed to 50 and 100 ppm H_2S increased by 0.7°C and then returned to normal levels after four days.

This trend correlated with plasma cortisol levels which increased initially but were near normal after four days of exposure. Consequently, some relationship involving cortisol synthesis, plasma corticoids and fever was suggested. The greatest single observation with exposure of goats was eye irritation. At 50 ppm, lacrimation was usually evident after 24 hours of exposure, while exposure to 100 ppm always produced lacrimation and usually eye injuries after 24 to 48 hours. By this time conjunctivitis was readily evident followed by partial loss of sight. Once removed from the H_2S, the eye epithelium lost its scarred appearance and sight apparently returned after three to four days. In general, eye irritation in the cows exposed to 20 ppm H_2S was much less severe, evidenced only by some slight lacrimation during the three-week exposure period.

Chronic poisoning for the most part has affected highly productive animals (97). During exposure of three lactating dairy cows for three weeks to 20 ppm H_2S, average milk production decreased by 1.24 kg/cow/day, but this was not statistically significant (58). The same exposure did not affect feed intake during either the gas- or post-gas intervals as compared to the pre-gas interval. Water was offered ad libitum. In the goats

exposed to 10 ppm H_2S, there was no statistically significant difference between gas- and pre-gas periods for feed and water consumption but, on the first day of exposure, feed and water decreased by 20 and 63% respectively. Intake resumed to normal levels thereafter. Exposure of goats to 50 ppm H_2S did not decrease intake until day 2 of gassing when feed and water consumptions dropped by 60.5 and 64% respectively. In this case, decreased feed intake for the remaining two days of exposure was maintained while water intake showed trends of increasing. The goats exposed to 100 ppm H_2S decreased feed intake by 37% on day 2 of exposure and increased slightly on days 3 and 4. Water intake was not significantly different during exposure- and pre-exposure periods. However, on day 2 of gassing, water intake decreased by 25% before resuming near normal levels. Hays (58) suggested that possibly the mechanisms controlling the desire to consume food and water are much different at lower levels compared to higher levels of H_2S. Observations indicated that the goats exposed to 50 ppm and 100 ppm H_2S decreased urine formation and at times no urine was collected at all. However, whether the urine decrease was because of a decrease in water intake or vice versa was never ascertained.

4.3 Accidents Involving Humans.

Incidents involving fatalities of humans from manure gas poisoning have occurred. One case report revealed that two farm workers at Red Deer, Alberta, were overcome when they entered a manure tank that was being pumped out (44). Similar cases have been reported (14,82) in which death has resulted, usually from entering partially-emptied slurry tanks. Non-fatal accidents have occurred by opening sluice gates in enclosed spaces (22) or entering buildings to investigate the abnormal behavior of animals already being affected by toxic gases (93). In this latter incident, both

men involved reported that there had been sharp odor and their eyes and noses became irritated. One of the men fell to the ground in a moment of faintness. Both had difficulty in breathing. Recovery was rapid following escape from the barn, but the victims complained of severe headaches throughout the day.

5. Summary and Assessment of Response Criteria.

5.1 General Discussion.

Confinement intensifies no animal-environment interactions more than those involving air-environmental factors (37). Catcott (32) reviewed the literature on gaseous pollution as affecting animals and concluded that the effect of specific pollutants is complicated by environmental factors. Hence, he suggested that the ideal experimental approach is a process of initial field observations, followed by specific experimentation under controlled conditions. Regarding air pollution diseases in farm animals, Lillie (79) advised that diagnosis should include the identity and concentration of pollutants in the area, a comparison of the clinical and pathological symptoms of affected animals with those of the same species given a similar dosage level in the laboratory, a comparison of the overall performance of the animals in affected and unaffected areas, a thorough investigation of the structural changes within the animal body, and the degree of toxicity (acute versus chronic). Although this approach appears highly desirable,it may be rather idealistic in view of the present state of the art. Research investigators have been cautioned (79) not to rely too much on some of the published results relevant to air pollution, because many of these results may not be true or may be misleading. In fact, one investigator termed some of the results facts and fables (105). Differences in data obtained in field and laboratory environments may be attributed to interactions of many factors necessary to produce critical physiological

and pathological situations (32).

At present, environmental engineers, veterinarians and physiologists are searching for an index, or combination of indices, that might measure the effects of exposure to practically encountered levels of manure gases on cattle and other classes of livestock. A number of climatic stress indices for domestic animals have been reported (78) in which the effect of given atmospheric conditions is assessed in terms of some single measurable animal reaction. All of these measures are crude and deal only with partial and selective manifestations of the overall condition. Unfortunately, the more one seeks to refine them, the more the inherent philosophical difficulties become apparent (78). Judging the effect of the cowshed environment can be both deceptive and inexact (45). Comparable results and definite conclusions can be drawn only from a study of both the animal's production and state of health. The state of health, like production, is regulated by numerous different factors, many of which affect both. Knowledge of these factors,however, is too incomplete to allow reliable conclusions to be drawn from consideration of one aspect only.

This review has shown that when studying animals to ascertain the extent of the manure gas problem, the application of a systematic and consistent examination procedure is essential to attain useful results. Three broad sets of variables, adopted from Lee (78) should be considered:

1) Environmental conditions.

Standardization of methods for measurement of specific air pollutants and demonstration of their accuracy are essential to permit measured gas levels to be correlated with effects on animals. In addition to intensity, the effects of exposure depend considerably upon the duration

of that exposure. Strict control of other aspects of the environmental complex (14) is essential to minimize possible masking or misinterpretation of experimental results.

Controlled environment chambers for animal research have been described (62,94) and mandatory requirements in the design and installation of animal inhalation exposure chambers set down (62). Compliance with these requirements will help investigators avoid some of the common difficulties and sources of error in their research.

2) Individual characteristics.

Examples of factors that must be considered within this set of variables are: species, breed and type, age, size, body weight, sex, metabolic state, nutrition, degree of activity, volume of breathing, rate of circulation, derangement and disease, and inherent individual variability.

3) Criteria of effect.

Many difficulties are associated with attempting to distinguish between toxic and sensory effects of manure gases in farm animals. Overt behavior as well as objective signs (tears, inflammation of eyes and mucous membranes) might indicate a sensory response, whereas, the systemic toxic effects would consist of chemical and morphological changes of tissues indistinguishable in general from those observed in humans (79). In discussing the manifestations resulting from exposure to NH_3 and/or H_2S, a more desirable approach may be to consider them on the bases of 1) production and performance data, and 2) state of health.

One must realize that this is merely an arbitrary distinction and in any case of exposure both aspects often may be affected by the same factors. In view of this distinction, production and performance data encompass growth, productivity and reproduction while the state of

health is measured by both general and clinicopathologic conditions, and incidence of disease.

Thus, the methods and criteria outlined as follows may be useful in evaluating and elucidating the effects of H_2S and/or NH_3 on cattle. At this point there will be no attempt to interpret any of the criteria listed, as this has been covered in preceeding sections of the review. Furthermore, there is no intention to provide collection or analysis procedures for the data and methods summarized. Rather, the purpose is to provide a checklist-type of guide to broad areas that might be considered in research investigations. Any effects of NH_3 and H_2S given are from published data cited in Section 4 of this review; as such, they are not necessarily contended to be reliable nor encompassing - they are intended merely to serve as general examples and plausible comparisons.

5.2 Production and Performance Data.

Production and performance factors are emphasized by their direct bearing on the crux of the cattle industry - economics - and, hence, may provide the most obvious changes and be of greatest concern to the stockman and researcher alike.

5.2.1 Growth Factors.

NH_3 and H_2S: inappetence (decreased feed and water intake), reduced growth rates and live-weight gains.

5.2.2 Productivity.

NH_3 and H_2S: decreased feed conversion efficiency, decreased lactation, teat injuries, loss of flesh, reduced carcass quality.

5.2.3 Reproduction.

NH_3: increased age at sexual maturity.

H_2S: abortions, excessive hemorrhaging with calving.

5.3 State of Health.

5.3.1 General Condition and Appearance.

NH_3 and H_2S:

1) unhealthy appearance: dull, rough coat; lameness.

2) evidence of pain, discomfort or distress: frequent lying down and getting up, switching, general uneasiness, pressure damage where cows rest on stall work to relieve weight on their feet, unnatural stance.

3) overt behavior: listlessness, standing with head lowered, increased sneezing and coughing, head shaking and jerking, slobbering, eye inflammation and lacrimation, keeping eyes closed, rubbing eyes, photophobia, acting blind, frequent defecation or scouring, infrequent urination, muscular spasms, staggering, convulsions, coma.

Included in this assessment of the general state of health should be an attempt to estimate the tolerance and acclimatization period to the existing level of gas(es). Records also should be made of any noted increase in the incidence of respiratory disease and secondary infections. e.g. chronic cough, pneumonia, pulmonary emphysema, calf diptheria, bronchitis, pleurisy.

5.3.2 Gross Pathology.

NH_3 and H_2S:

1) eyes: excessive lacrimation, conjunctival irritation, corneal opacity and erosion, blindness.

2) nose and mouth: excessive secretions, frothing at mouth, epistaxis.

H_2S: • Cyanosis (lower abdomen), hemorrhaging (subcutaneous, subpleural, and in horn of hoofs), cracks and infection in hoofs, loss of motion in rumen and intestines.

5.3.3 Physiological Examination and Observation.

1) Respiration changes.

NH_3 and H_2S: respiratory rate, respiratory depth, respiratory pattern (short, irregular), dyspnea, shallow breathing.

NH_3: respiratory compensation may correlate with changes in blood pH (minimum at high pH, maximum at low pH).

Comment: Respiration rate, as such, may not necessarily be a good means for quantifying respiratory efficiency (58). Instead, the volume of breathing (60), although more inconvenient to measure, may provide a more accurate assessment.

2) Body (rectal) temperature.

H_2S: increase.

Comment: Fever in mammals is very common with the inflammatory process associated with infection, antigen-antibody reaction or necrosis (58).

3) Cardiovascular changes.

H_2S: increased heart rate (tachycardia), increased pulse rate and blood pressure.

5.3.4 Hematology.

Clinicians generally recognize that appropriate laboratory tests can reveal many things that are beyond the scope of a physical examination. Several hematological characteristics are studied routinely as diagnostic aids in cases of human and animal pathology. Correlations of some hematological values with adaptability to adverse environmental conditions have been reported (54). The use of tests on blood constituents may well have a future in making a diagnosis or prognosis, acting as a guide to

rational therapy, and providing a deeper understanding of the fundamental processes of manure gas poisoning. At present, the most serious limitations to the full utilization of laboratory data are at the stage of interpretation (89). Advantages of blood analysis (89):

i) blood is the easiest tissue to sample by biopsy without harming the animal,

ii) a single sample gives a static picture,

iii) a series of samples gives a dynamic picture of sequential physiologic and pathologic changes occurring during the sampling period.

1) Morphology.

NH_3: Hb (initial elevation, ultimate suppression)
PCV (initial decrease, ultimate increase)

H_2S: low Hb (anemia)
low total white cell count, increased blood coagulation time.

2) Blood Chemistry.

NH_3: blood pH, NH_3 and bilirubin decrease and SGOT increases (liver malfunction); red cell [Na] (decrease) and [K] (increase); analysis of other NH_3 by-products.

H_2S: CO_2 content (decrease), albumin/globin ratios (decrease), plasma cortisol changes.

Comments: Changes in blood chemistry which may occur during stress include (114):

i) Lipids: after an initial decline, cholesterol, free fatty acids, and triglyceride levels increase.

ii) Ions: Na and Cl decrease during shock phases of stress, but rebound to above normal during chronic stress; the reverse occurs

with K. Ca levels rise with chronic stress.

iii) Carbohydrates: gluconeogenesis increases due to increased catabolism of protein and fatty tissue.

iv) Nitrogenous compounds: non-protein nitrogen, urea, and amino acids increase, but total protein decreases.

Changes in blood chemistry itself can result in pathological conditions which have been termed 'diseases of adaptation' (114).

5.3.5 Post-mortem Examination and Histopathology.

1) Respiratory tract.

NH_3: pulmonary edema, congestion, hemorrhage, ciliary loss, decrease in goblet cells, increased thickness of tracheal and nasal epithelium, necrosis.

H_2S: lung edema, emphysema, pulmonary hemorrhage, hypostatic congestion, lungs dark in color.

2) Other tissues and organs.

H_2S: degeneration and hemorrhaging of muscles and visceral organs, brain edema, back muscles almost black in color, offensive odor to carcass, intestines acutely inflammed - gastrointestinal ulceration has been termed a 'disease of adaptation' (114).

3) Eyes.

NH_3 and H_2S: opacity, thickening and erosion of cornea, cellular reaction, absence of Bowman's membrane.

4) Skeleton.

NH_3: weak ribs and long bones (Ca deficiency resulting from 'environmentally induced nutritional stress' (34)).

Comment: The value of necropsy or a post-mortem examination is questioned for the following reasons:

i) Inconclusiveness of results: Several researchers (7,38,42,93, 100,118) have noted that damage to the respiratory tract and visceral organs from low concentrations of NH_3 and H_2S may be undetectable with gross or histopathological examinations.

ii) Expense: the scheduled sacrifice of cattle during a test period in an attempt to correlate changes with exposure time could prove extremely costly and consequently, even prohibitive. Post-mortem following natural death from exposure is, of course, a different case and is to be encouraged.

iii) Such examinations require a qualified pathologist, specialized equipment and facilities, and often tedious procedures. Many research situations may be unable to meet these requirements.

5.3.6 Microbiology.

NH_3 and H_2S: increased bacterial counts from tracheal and nasal swabs.

Comment: Identification of potentially pathogenic microflora that may become clinically evident if bodily resistance is undermined by gassing could be valuable in treatment or prevention of disease.

5.3.7 Infectious Challenge.

NH_3 and H_2S: Damage to the respiratory tract which might otherwise pass undetected may become apparent when the animal is challenged with airborne infectious microorganisms (7,46,48).

Comment: Availability of appropriate test animals and experimental quarters may limit wide applicability of this method. Animals should be pathogen free or at least raised under identical conditions before any results can be considered reliable.

6. Conclusion.

Clearly, the differentiation of reduced livestock performance

caused by manure gases from effects caused by other environmental stresses (nutritional disorders, poor husbandry practices, parasitism, airborne pathogens, non-atmospheric poisons, temperature, humidity, light, sound, and others)(29,79) can be extremely difficult. Little information is available on the question of tolerance and adaptation because the procedures involved in studying the effects for long periods and at various levels of exposure are expensive and tedious (95). This can be dealt with in part by making full use of facilities belonging to stockmen who also are seeking new knowledge and who are willing to cooperate in research projects.

No single discipline, let alone any one person, is likely to be cognizant of all the areas involved in air pollution. Measurement and resolution of the numerous effects associated with the manure gas problem will require integrated research involving several disciplines; a team approach among the agricultural engineer, the veterinarian, the animal scientist and others as required will be necessary. How best to effect this form of research is a problem in itself (29).

III. OBJECTIVES

The review of literature indicated that there is immediate concern as to the adverse effects of sub-lethal concentrations of manure gases on animal health and performance over the short or long term. While manure gas poisonings of cattle have been attributed mainly to H_2S, suggestions have been made that other gas components may play an important role, with the combination of H_2S and NH_3 possibly being of most significance. However, definitive experimental evidence to support this contention appears to be lacking.

This study, therefore, was undertaken to investigate the response of calves exposed for a continuous period of seven days to controlled levels of H_2S and NH_3, alone and in combination. The following criteria were used to evaluate response:

1. Clinical observations. A sequence of clinical and gross pathologic examinations, as well as evaluations of behavior and general condition, were conducted in assessing the overall state of health and estimating tolerance and adaptation periods. Clinical symptoms were to be compared with those reported as characteristic for humans and animals exposed to H_2S and NH_3.
2. Feed and water consumption. Feed and water consumptions were recorded as an index of production performance and general well-being. Liveweight gain was included as part of the production data.
3. Respiration rate. Respiration rate was taken as a means to quantify changes involving the respiratory system and to compare responses with those reported in the literature.

4. Rectal temperature. Rectal temperature was measured as an index of general stress and to detect possible inflammatory or neural effects. Furthermore, body temperature was to serve as an indication of respiratory disease and to furnish a guideline to determine whether treatment was required.

5. Blood constituents. The blood was studied as a potentially convenient adjunct to the diagnosis and elucidation of manure gas poisoning. Although the review of literature indicated that changes in blood constituents may be expected, pertinent reports were extremely scarce and lacking in general agreement. Hence, the most efficient approach appeared to be to subject blood samples to a battery of tests. The laboratory information then could be evaluated for possible correlations with the toxic effects of H_2S and NH_3. In this light, measurements were chosen to provide a comprehensive range of clinical data. Included were tests for sulfhemoglobin and NH_3, a complete blood count, a total biochemical profile (12 constituents), and analysis of blood gases (pO_2, pCO_2) and hydrogen ion concentration (pH).

IV. MATERIALS AND METHODS

1. Experimental Design.

The experiment was designed to expose calves to NH_3 and H_2S gases, alone and in combination. The controlled exposures consisted of three different levels of each gas: zero, low and high. A quantitative description of the factor levels to be maintained during the gas-exposure period is included in Table 6. Corresponding designations for the treatment combinations are also shown.

Nine animal inhalation exposure chambers were utilized such that each chamber, housing two calves, received a different treatment combination. A second replication with another 18 calves completed the experiment. Each replicate comprised three one-week periods during which calf responses were evaluated. A pre-exposure (control) period of seven days preceded seven continuous days of gas infusion which in turn were followed by a seven-day post -exposure period.

TABLE 6. GAS EXPOSURES AND TREATMENT DESIGNATIONS.

Factor	Levels		
	zero	low	high
H_2S	0	20 ppm	150 ppm
NH_3	0	50 ppm	150 ppm
Designation	0	1	2

	Treatment Combinations								
H_2S	0	0	0	1	1	1	2	2	2
NH_3	0	1	2	0	1	2	0	1	2

2. Facilities.

2.1 Livestock Environmental Engineering Laboratory.

This study was conducted in the Livestock Environmental Engineering Laboratory on the research farm of the Department of Agricultural Engineering, University of Alberta, located at Ellerslie, Alberta. The 100 x 40-ft facility has been described previously (85). Situated between the livestock weigh room and the feed room is the 80 x 40-ft fully slatted livestock area. The pressurized ventilation system, incorporating a centrifugal fan at each end of the building, forces outside air into the livestock area through central ducts running at a height of 11 ft above floor level.

Nine identical animal exposure chambers, situated within the livestock area of the laboratory, were used for the calf exposure trials. The basic design* and construction** of the experimental unit had been completed prior to the author's involvement with the layout.

The arrangement was such that five chambers were located along the west side of the area and faced the other four chambers and an instrument room across a center aisle (Figures 1 and 2). This aisle provided space for a feed storage bin, the gas cylinders, and a walkway of plywood sheets laid over the slats around the perimeter of the aisle.

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* Design recommendations by Prof. J.B. McQuitty, Dept. of Agric. Engineering, University of Alberta, Edmonton, Alberta.

** Construction supervised and assisted by Mr. A. Blecha, Technician, Dept. of Agric. Engineering, University of Alberta, Edmonton, Alberta.

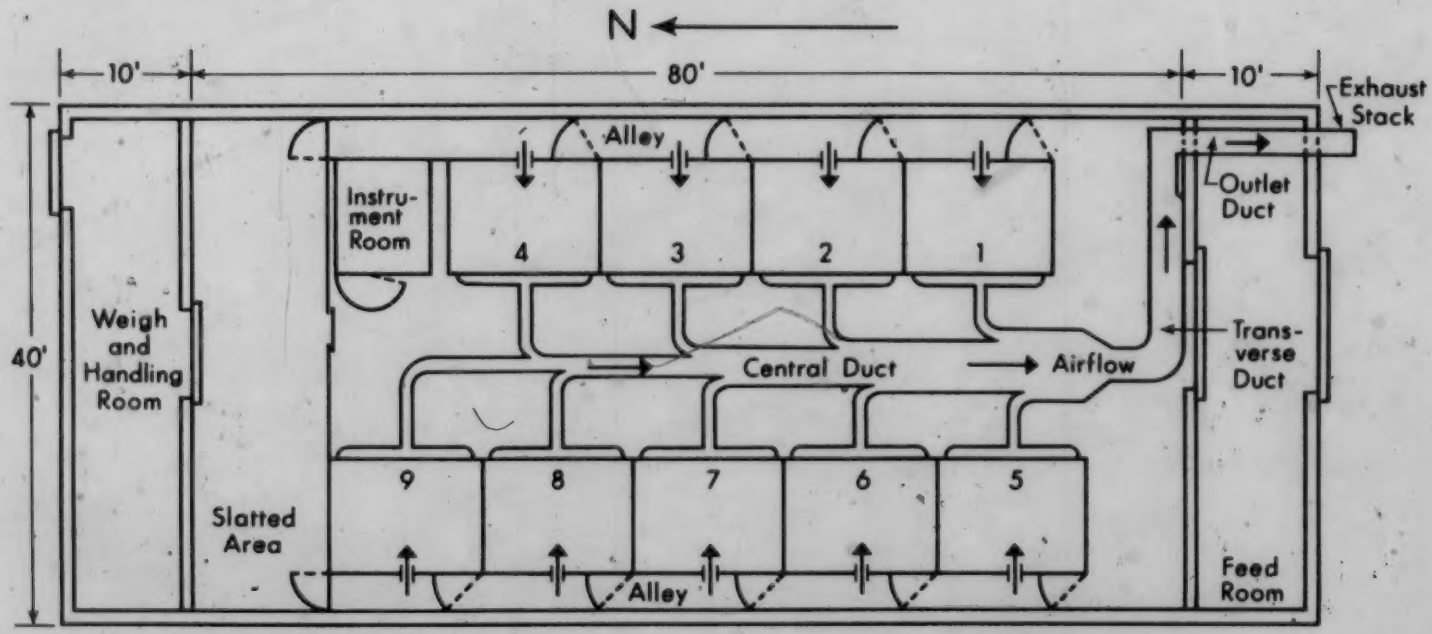

Figure 1. Plan view of exposure complex showing arrangement of animal chambers and exhaust ductwork.

The exposure complex was designed to provide the animal chambers with a self-contained ventilation system. All ductwork was constructed using masonite, plywood, and galvanized steel sheeting fastened to wood framing members. The entire system was suspended from the steel lattice trusses of the environmental laboratory.

Exhaust air was collected in a plenum built across the front of each chamber. Individual ducts, perpendicular to the plenums, extended to a common exhaust duct running along the centerline of the building (Figure 2). This central duct, 9 in. deep and of increasing width, continued to the wall partitioning the livestock area and the feed room where it joined a transverse duct paralleling the inside of the partition. The transverse duct, measuring 32 in. wide and 23 in. deep, met an outlet duct that connected to a 10-ft exhaust stack rising up the outside end wall (Figure 3). Both the outlet duct and the insulated stack measured 24 in. wide by 32 in. deep in cross-section and had been constructed as part of a previous cattle housing study (52).

Because initial tests had shown that changes in wind velocity and direction resulted in fluctuating ventilation rates through the chambers, a cowl and skirting* were added to the top of the stack. This apparatus tended to stabilize fan capacities and hence somewhat alleviated the task of maintaining uniform gas concentrations within the exposure chambers.

2.2 Animal Inhalation Exposure Chambers.

2.2.1 General Construction.

One exposure chamber was designed and built as a prototype for

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* Designed and constructed by R.C. Bacon, Technician, Dept. of Agric. Engineering, University of Alberta, Edmonton, Alberta.

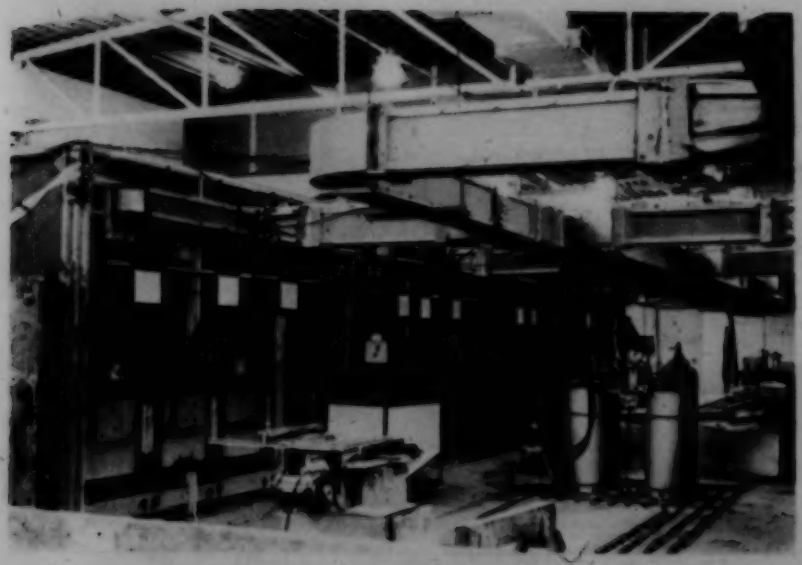

Figure 2. View down center aisle showing Chambers 1 to 4 and adjoining ductwork.

Figure 3. Exhaust stack and cowl apparatus.

the eight others that followed. A floor of 3/4-in. plywood sheeting was supported approximately 1 ft. above the top of the slats on wooden joists. The rectangular chamber measured 8 x 12-ft wide and 8 ft high. The walls were formed with 4 x 8-ft, 1/8 in. thick, masonite sheets nailed to the inside of nominal 2 x 4-in. spruce studs. A 4 x 12-ft double layer of 4-mil polyethylene film comprised the front half of the ceiling to allow light from the sky panels and electric lights of the laboratory to enter the chamber. The other half of the ceiling consisted of masonite sheets nailed to 2 x 4-in. joists. All joins and cracks in the floor, walls and ceiling were thoroughly sealed with caulking compound.

The chambers originally were constructed to house four 150-300 lb dairy calves and one sheep in individual tie stalls spaced across the front section. Each calf stall measured approximately 2 1/2-ft wide while the sheep stall was about 2-ft wide. All stalls were lined and partitioned with 3/4-in. plywood sheets and measured 4-ft high by 5-ft long, including the manger area. Each manger was equipped with a built-in water pail and a removable feed box.

The unavailability of suitably small dairy calves at the beginning of the experiment forced the purchase of beef-type calves. These animals were larger than desired and as a consequence the chambers had to be remodelled. Subsequent alterations essentially provided two 5 x 8-ft box stalls for the accommodation of two calves per chamber, as shown in Figure 4. The sheep stall was simply boarded across the back and each calf stall now contained two mangers (Figure 5).

A large 3 1/2 x 5 1/2-ft door of 3/4-in. plywood was featured at the back corner of each chamber. The door swung outward to butt

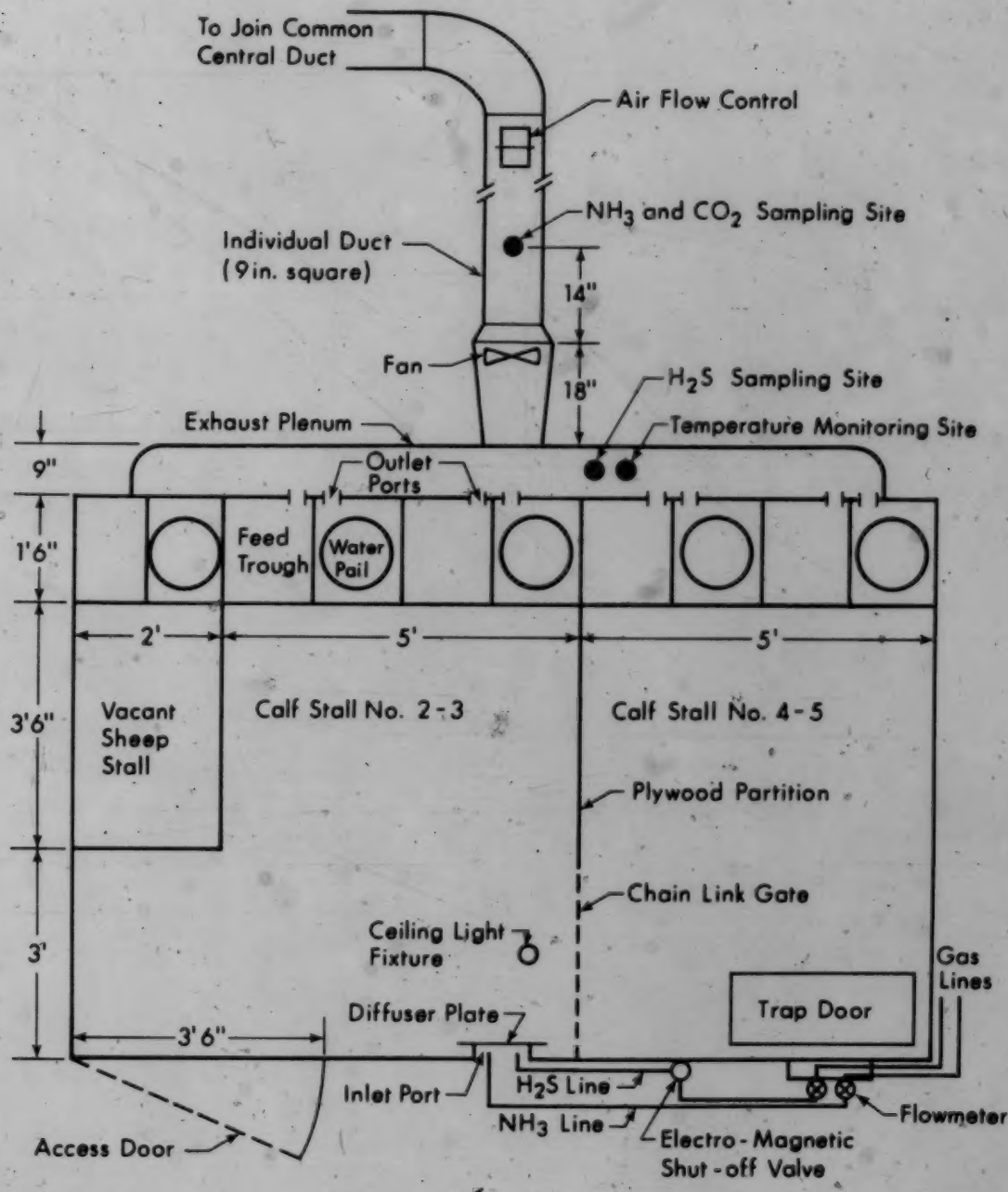

Figure 4. Plan view of an animal exposure chamber.

against the side wall of the laboratory, allowing convenient access to the stalls from the alley. Latches and weatherstripping ensured a tight seal when the door was closed.

Plexiglass observation windows were installed in the front of the chambers directly over each manger (Figure 5). A broad inside view was also possible through the ventilation inlet port located in the back wall of the chamber (Figure 10). A 100-watt bulb, in a ceiling fixture located between the stalls, provided ample light for observation purposes. The light switch was accessible from outside the chamber.

Finally, a trap door, measuring slightly less than 2 x 3 ft, and set in one corner of the chamber floor, allowed excreta to be shovelled directly into the underlying pit.

2.2.2 Ventilation System.

Each exposure chamber was ventilated separately by means of a direct-drive propeller fan[a] secured within the frontal ductwork (Figure 4). This method of exhausting air maintained a slight negative pressure within the chamber to prevent any outward leakage of gases.

Air from the livestock area was drawn through a 9 in. square inlet port centered in the back wall of the chamber, at a height of 4 ft from the floor. A Plexiglass diffuser plate, projecting inward approximately 3 in., overlapped the inlet area and served to distribute the inflowing air evenly throughout the chamber (Figure 10).

Five pairs of 3 1/4-in. diameter holes, located 1 ft below the ceiling and spaced across the front wall, functioned as outlet ports (Figure 6). Sliding wooden plates mounted over these ports on the outer wall enabled ventilation rates to be adjusted for each chamber. For the

[a] Dayton model 3M230, 1500 rpm, thermally protected.

Figure 5. Interior view of box stall showing manger facilities and observation windows.

Figure 6. Interior view of ventilation outlet ports (3 1/4-in. diameter) located in front wall of chamber.

purpose of this experiment, the pair of ports above the vacant sheep stall were closed off, thus providing equal outlet areas over both calf stalls.

From the plenum, exhaust air converged into a short length of ductwork housing the exhaust fan (Figure 7). The dimensions of this and adjacent ductwork, being 9 in. square in cross section, were based primarily on the minimum area required to accommodate the fan. Each individual frontal duct subsequently joined the common central duct via a long radius 90° elbow.

2.2.3 Gas Infusion System.

Both NH_3 and H_2S gas were supplied in cylinders chained to a stand located in the center aisle (Figure 2). One pair of cylinders each contained 100 lb of anhydrous NH_3 under an initial pressure of 114 psig at 70°F[b], while the other pair each contained 170 lb of H_2S under an initial pressure of 252 psig at 70°F[c]. Each cylinder pair was connected to a two-stage corrosion-resistant, pressure regulating valve[c], as shown in Figure 8, with 1/4 in. aluminum tubing and Swagelok[d] fittings. From the regulator delivery gauges, 3/8 in. polyethylene tubing ran through size 50 psig electro-magnetic shut-off valves[e]. These two-way safety valves were employed to block the gas supply lines in the event of an electrical failure.

The gases were distributed separately via two manifolds sealed

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b Canadian Liquid Air Ltd., 8615 Stadium Road, Edmonton, Alberta.

c Matheson of Canada Ltd., P.O. Box 322, 530 Watson Street East, Whitby, Ontario.

d Edmonton Valve and Fitting Ltd., 9639 - 62 Ave., Edmonton, Alberta.

e Cantech Controls Ltd., 10567 - 111 Street, Edmonton, Alberta.

Figure 7. Chamber exhaust fan (bottom section of duct removed).

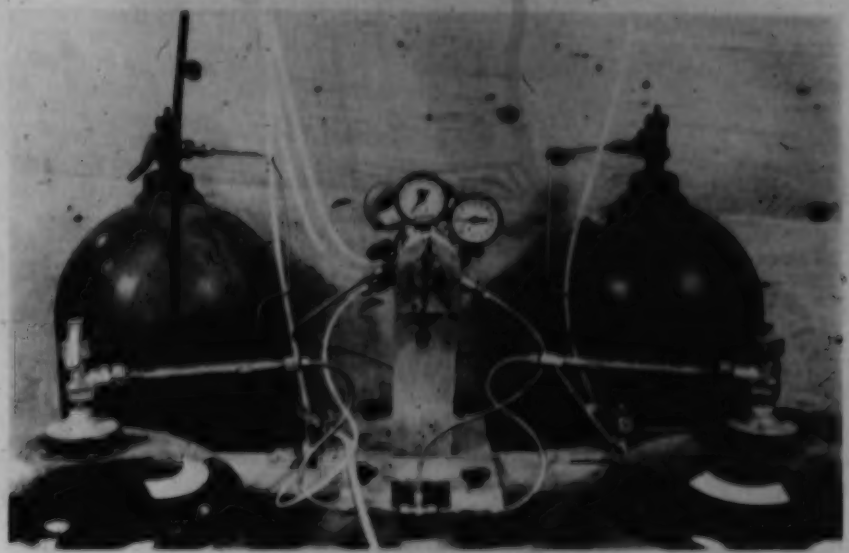

Figure 8. Ammonia cylinders (foreground) and hydrogen sulfide cylinders (background) with gas fittings and pressure regulators.

on the top of the central duct. Each manifold, fabricated from 1 in. diameter seamless stainless steel, was appointed with nine outlet fittings. From the manifold outlets, 3/8 in. polyethylene tubing led to individual flowmeters[f] mounted on the back wall of each chamber (Figure 9). The flowmeters, with needle valves, provided the means of measuring and controlling the rate of gas delivered to each chamber.

Two infusion tubes, consisting of 1/4 in. aluminum tubing bent near the end at right angles and suspended in the inlet port, introduced the gases directly against the diffuser plate to facilitate mixing with the incoming air (Figure 10). In the initial setup, both gases had been teed together to be infused through a single tube. However, when H_2S and NH_3 were mixed in the common line, a solid, yellow-colored deposit began to form and tended to plug the lines.

Immediate attempts to have the deposit analysed chemically were unsuccessful, and the samples deteriorated with time. Furthermore, personal inquiries and a brief search of the literature failed to provide any enlightenment as to the cause or nature of the reaction. From a visual and olfactory appraisal, the deposit was presumably a sulfur compound, perhaps involving NH_3 or some contaminants that had accumulated in the lines. Notably, only lines in which H_2S and NH_3 had been mixed formed deposits, and individual lines for each gas eliminated any further complications of this sort.

2.2.4 Safety Feature.

A gas-flow control circuit was installed for each exposure chamber. The concept consisted of an air-flow control[g] (Figure 11)

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[f] Brooks Instrument Division, Emerson Electric Canada Ltd., P.O. Box 150, Markham, Ontario.

[g] Stapleton Instruments Ltd., 6212 Davies Road, Edmonton, Alberta.

Figure 9. Gas flowmeter system mounted on outer back wall of chamber.

Figure 10. Inlet port in back wall of chamber with gas infusion tubes and Plexiglass diffuser plate.

wired in series to an electro-magnetic shut-off valve[e] positioned in the gas line. When the common line was eliminated, as mentioned earlier, the H_2S line was connected through the electric valve while the less-toxic NH_3 line was by-passed.

The air-flow control switches were secured on top of the individual ducts with the paddles positioned downstream from the fans and perpendicular to the direction of flow. The control detects air flow or the absence of air flow in ducts, responding only to the velocity of air movement. An opposing spring force sets the cfm flow required to activate an internal switch. To realize satisfactory operation at the relatively low duct velocities, 6 x 8 in. paddles were fashioned from 28 gauge aluminum sheeting[h] and substituted for the smaller standard paddle. The circuitry was such that H_2S could be infused only when the fan was operating and hence maintaining the electric valve in the H_2S line at the back of the chamber in the open position.

2.3 Safety Equipment.

For emergency protection, two sets of self-contained breathing apparatus[1] (Figure 12) were mounted at strategically accessible locations in the laboratory. In addition, H_2S detector ampoules[j] were placed throughout the building and at times worn by workers to indicate if excessive exposure had occurred. These ampoules were also helpful in detecting leaks in the H_2S lines and ductwork.

To test the air, an ampoule is crushed to saturate the cotton

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h Central Engineering and Electronic Stores, Chemical-Mineral Engineering Building, University of Alberta, Edmonton, Alberta.

1 MSA Canada, 14608 - 116 Ave., Edmonton, Alberta.

j Safety Supply Co., 6120 - 99 St., Edmonton, Alberta.

Figure 11. Air-flow control switch and paddle (removed from duct).

Figure 12. Self-contained safety breathing apparatus.

covering with a reactive solution. Comparison with a color chart after one minute indicates whether more or less than 10 ppm (TLV)* is present by the color change to a shade of brown. The solution remains active for approximately six days after the ampoule is crushed.

3. Environmental Measurements.

3.1 Gas Analysers.

3.1.1 Ammonia and Carbon Dioxide.

Ammonia and CO_2 levels were measured by two Beckman Model 315A non-dispersive infra-red analysers[k], designed specifically for each gas (Figure 13). These instruments operate on the principle of measuring the differential absorption of infrared energy between a sample cell and a reference cell. As a chopper blocks and unblocks the infrared beam to each cell, a diaphragm, in a detector filled with vapor of the component of interest, pulses because of pressure differentials between the compartments on each side. This pulse is converted electronically to a per-cent deflection on a voltmeter scale. The corresponding concentration of the gas sample is determined from a calibration curve. Operating details have been discussed by Brannigan (23).

Start-up procedures included calibrating the analysers with a zero gas standard[b] and appropriate span standards[l]. Prior to recording gas levels during the experiment, both analysers were routinely checked against the calibration standards.

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* Threshold limit value.

k Beckman Instruments, Inc., Process Instruments Division, Fullerton, California.

l Alberta Oxygen Ltd., 5834 - 87 Street, Edmonton, Alberta.

Samples were drawn through a 3/8 in. polyethylene line from the NH_3 and CO_2 sampling site in the top of each chamber outlet duct (Figure 4) by means of a self-contained vacuum pump. A wall-mounted glass manifold in the instrument room (Figure 14) collected the nine lines from the sampling sites. A series of Teflon valves incorporated in the manifold permitted samples to be successively and expeditiously isolated and fed through the analysers. Airflow was controlled to each instrument by separate flowmeters.

Although gas levels normally were recorded every morning and afternoon for all periods, the NH_3 levels often were checked more frequently during the gas exposures. Mean daily NH_3 concentrations were calculated from all measurements taken within a day. Carbon dioxide was not infused; measurements were taken only to record background levels.

3.1.2 Hydrogen Sulfide.

Finding a suitable and accurate H_2S analyser proved to be a major problem. Initially, an RAC Model G2-SER-3915 automatic sampler[m] (Figure 13), owned by the Department of Agricultural Engineering, was installed for H_2S analysis purposes. This unit uses the following basic sampling procedure: 15 cfh of air is drawn past a section of H_2S-sensitized (lead acetate) tape and exhausted through a pump. However, the sampler is designed to automatically record gas concentrations to a maximum of approximately 0.4 ppm* H_2S. The levels to be analysed during this experiment ranged from 20 to 150 ppm.

In efforts to overcome the design limitations, pure nitrogen gas[b] was metered into the air stream as a sample was drawn into the

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[m] Research Appliance Company, Route 8, Allison Park, Pa., 15101, U.S.A.
* parts per million by volume.

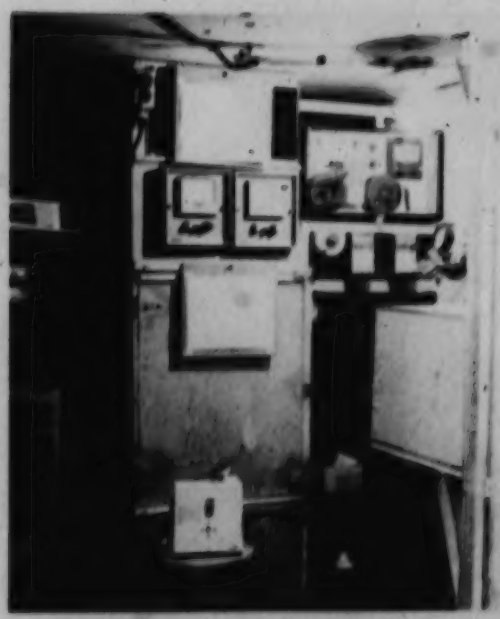

Figure 13. Interior view of instrument room - thermocouple recorder (left), NH_3 and CO_2 analysers (top center), Bullard H_2S monitor (bottom center), and H_2S automatic sampler (top right).

Figure 14. Ammonia and carbon dioxide gas sampling manifold in instrument room.

analyser. This technique was intended to dilute the samples to within the instrument's operating range. However, various attempts to achieve satisfactory results failed, and the analyser finally was abandoned.

The second instrument acquired was a Bullard Model 2103 H Hydrogen Sulfide Gas Monitor[n] (Figure 13), designed to detect levels between zero and 200 ppm with an accuracy of ± 3% of actual reading. The heart of this monitor is a solid state electrolytic cell gas sensor. In the presence of H_2S, the gas molecules dissolve into the solid state material, conducting electricity that is collected by electrodes and used to drive a meter.

The unit, although tested by several sampling methods, had to be rejected due to a lack of accuracy and repeatability, in addition to unstable meter drift*. A replacement model was also unacceptable for similar reasons.

The sampling method finally adopted for H_2S measurements utilized a Gastec Multi-Stroke Gas Sampling Pump and Gastec Detector Tubes[o]. The detection principle incorporates the reaction of H_2S with lead acetate to form brown-colored lead sulfide.

$$H_2S + Pb(CH_3COO)_2 \rightarrow PbS + CH_3COOH.$$

Thus, movement of H_2S through the detector material in the tube forms a coloration change which is quantified by reading the concentration at the interface of the stained-to-unstained reagent.

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[n] E.D. Bullard Company, Dept. IST, 2680 Bridgeway, Sausalito, California, 94965.

* Tested by the Laboratory Chemist, Industrial Health Services, 10523 - 100 Avenue, Edmonton, Alberta.

[o] Levitt-Safety Ltd., 5539 - 99 St., Edmonton, Alberta.

Hydrogen sulfide concentrations in the chambers were measured directly through a hole bored in the bottom of each exhaust plenum (Figures 4 and 15). Briefly, the procedure involved breaking the tips off each end of a detector tube that had been pushed through the bore of a rubber stopper, and inserting the stopper into the sampling hole. After placing the protruding end into the inlet of the pump, the plunger handle was pulled out, drawing a sample of air through the tube. The operator then waited two to three minutes, until staining stopped, before withdrawing the apparatus.

For measurement of the low and high H_2S levels employed in the experiment, different tube sizes were used. An extra low range (0.5 - 60 ppm) tube was suited to the 20 ppm concentrations, and required one pump stroke (100 cc) to produce a true reading. The 150 ppm levels were measured with a low range (10 - 120 ppm) tube by pulling one half of a full stroke and doubling the calibration scale reading to obtain the true concentration. The minimum accuracy of these tubes was guaranteed by the manufacturer to $\pm$ 25%, but trials on some tubes, with a known concentration of H_2S in nitrogen[b], indicated that the accuracies were within $\pm$ 10%. Repeatability with the tubes tested was most satisfactory.

Spot checks made prior to the gas-exposure periods found no measurable quantities of H_2S in the stalls. Therefore, H_2S levels were not taken routinely during the pre- and post-exposure periods. Concentrations were recorded regularly, at least twice daily, during the gas exposure periods. All measurements taken over a day were averaged to provide a mean daily H_2S level.

The Gastec unit, being the most mobile analyser, was used to check the distribution of gas within a chamber containing both calves.

Measurements of the H_2S concentrations taken at various heights between the floor and exhaust plenum indicated that the gas was distributed equally throughout the chamber. These results were accepted as confirmation that the sampling sites provided reliable measures of the gas concentrations existing at calf level.

3.2 Temperature Instrumentation.

Air temperatures were measured using copper-constantan thermocouples. Wet-bulb and dry-bulb thermocouples were provided in pairs for each chamber, with both junctions centered in the air stream through one hole in the top of the exhaust plenum (Figure 4). Three other thermocouple pairs monitored temperatures in the outlet duct, the inlet port of chamber 4, and the center aisle of the laboratory. The wet-bulb leads were covered by one end of a standard psychrometric sock, the other end of which extended into a plastic jar containing water (Figure 15).

The 24 thermocouples were wired into a Honeywell temperature recorder[p] situated in the instrument room (Figure 13). The recorder was equipped with a switching device that permitted either dry- or wet-bulb temperatures to be sequentially printed on chart paper. Temperature measurements were conducted twice daily at approximately 0800 and 1600 hr. These data provided a reasonable appreciation of the temperature extremes occurring throughout a day.

All thermocouples were individually calibrated by comparing readings to a sling psychrometer that had been previously calibrated using standard laboratory procedures (19). Although the air velocities

[p] Minneapolis-Honeywell Reg. Co., Brown Instruments Division, Philadelphia, Pa.

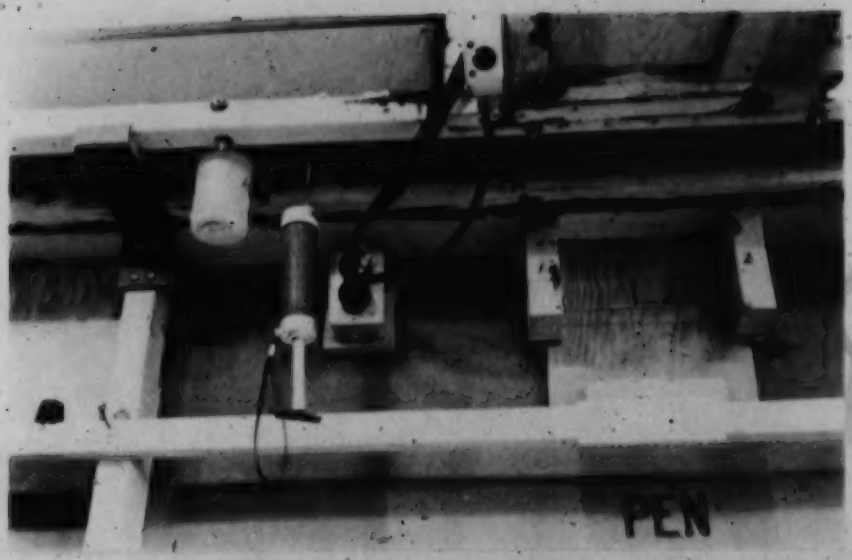

Figure 15. Gastec hydrogen sulfide detector apparatus in sampling position. (Wet-bulb reservoir located on left.)

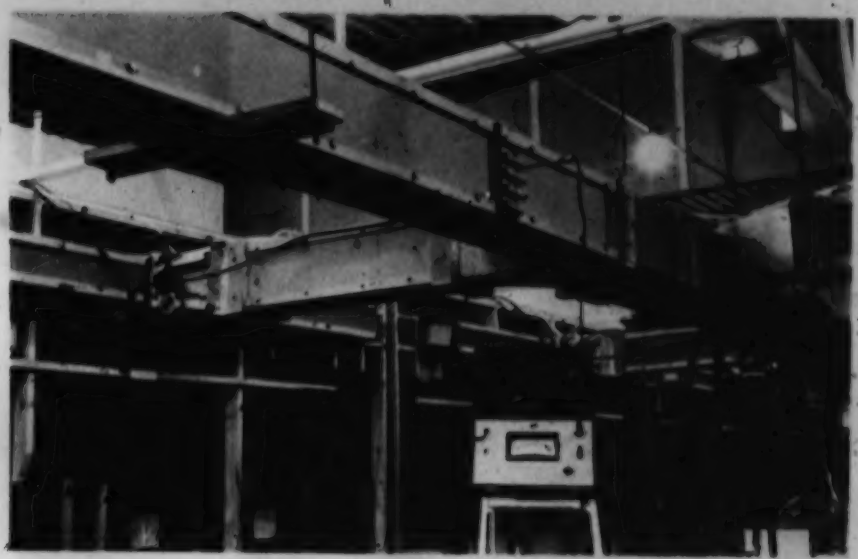

Figure 16. Hot-wire anemometer with probe in central duct.

over the bulbs were less than the 800 or 900 fpm considered necessary to ensure wet-bulb accuracies to within 0.5%, the slight wet-bulb depression can be ignored (71). Average relative humidities were determined for each day by entering a psychrometric chart[q] with the corrected mean daily dry-bulb temperatures and the corresponding corrected mean daily wet-bulb temperatures.

3.3 Ventilation and Gas Flow Rates.

The ventilation rate for chamber 9 was determined using a hot-wire anemometer[r] to measure the air-flow velocity in the first section of the central duct (Figure 16). Prior to use, the accuracy of the anemometer was checked in the test setup illustrated by Brannigan (23), using the procedure described by Jorgensen (71).

A cross-section of the 9 x 9 in. duct was divided into 25 equal squares. The velocity was measured at the center of each square by making successive horizontal traverses with the heat-sensitive probe. The 25 values then were used to calculate the average velocity in the duct. This procedure was repeated several times before arriving at a final average velocity.

With the outlet ports above the calf stalls opened fully, the average velocity was almost 260 fpm. This provided a chamber ventilation rate of approximately 145 cfm (72.5 cfm per calf), or just over 11 air changes per hour. Since the other chambers were adjusted to have outlet areas equal to that of the reference chamber, for all practical purposes the air-flow rate through each chamber was assumed to be the same.

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[q] Normal Temperature Psychrometric Chart, Carrier Corporation.

[r] Hastings-Raydist, Inc., Hampton, Virginia 23361.

Besides satisfying the ventilation requirements for the calves (30), the air-flow control switches functioned consistently well at this flow velocity. Furthermore, calculations indicated that with gas flowing at the desired rates, the flowmeter floats would be positioned near mid-scale - the ideal operating range for precise control.

Preliminary trials with the gas-infusion system indicated that flow rates through the meters were steadier at cylinder delivery pressures above 5 psig. Hence, the regulators were maintained to deliver gas at 10 psig during the gas-exposure periods. Initial air-equivalent settings of the gas flowmeters were calculated based on the chamber ventilation rates, as detailed in Appendix 1.

Three different tube sizes with an accuracy of $\pm$ 5% of maximum scale[f] were used to measure and regulate the infusion rates. Meters with capacities* of 140 cc/min., 1.2 scfh, and 2.0 scfh were installed for the respective levels of 20 ppm H_2S, 50 ppm NH_3, and 150 ppm H_2S and NH_3. Regular fine adjustments were necessary to maintain the gas levels within the desired ranges.

The corrosive action of H_2S on metal surfaces was evidenced during the experiment, and posed particular maintenance problems with some of the gas-handling equipment. Initially, a ceramic bronze filter[c] had to be installed in the inlet nipple of the H_2S regulator to prevent cylinder debris from entering the regulator and causing the valve seat to leak. Near the end of the second gas-infusion period, this filter became so plugged with a black deposit that the gas flow was impeded drastically. A replacement filter returned the system to normal.

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* Air-flows at 14.7 psia and 70°F.

During preliminary proceedings, corrosion of the stainless steel tubing in the H_2S cylinder hookup resulted in particles accumulating downstream in the lines and flowmeters. The replacement of all stainless steel tubing with aluminum tubing prevented further incidents of this nature.

After the first gas-exposure period, the lines were purged with nitrogen gas and the flowmeters were cleaned thoroughly. However, even with these precautionary measures, the surfaces of some of the stainless steel rotameter floats began to corrode and roughen noticeably during the second replicate. This caused the floats to behave rather unstably in the gas stream and, therefore, constant surveillance was necessary to prevent excessive fluctuations in the gas-infusion rates. Note that after the first replication, most of the flowmeters had to be switched to correspond to the gas treatment combinations randomly allocated to the chambers for the next replication. Hence, some meters were subjected to high flows of H_2S during both gas-exposure periods, while others may have been used for high H_2S levels only once. Because of this, the attention required by each meter varied.

4. Calf Management.

4.1 Adjustment Periods.

Calves for both replicates were purchased in separate lots through a livestock buyer* at the Edmonton Public Stock Yards. The first load of 27 steers arrived at Ellerslie on July 10, 1974. Although this load contained an assortment of breeds and sizes, the calves were predominantly beef types. Selection at the time of purchase was poor due

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* Don Danard Livestock Ltd., 127 Ave. - 52 St., Edmonton, Alberta.

to a lockout by local meat packers that resulted in a very limited turnover of stockyard cattle.

Upon arrival, each animal was weighed, ear-tagged, injected with vitamins A-D-E, and vaccinated for Blackleg and Infectious Bovine Rhinotracheitis (I.B.R.). Weights ranged from 298 to 542 lb, with the average initial weight per steer being 441 lb.

After six days in an outside feedlot, the calves were confined, three per chamber, in the pre-existing tie stalls. Although these stalls had been improvised with extension frameworks to hold the larger calves, a trial period of seven days indicated several stressing problems that demanded rectification before the project could proceed successfully. The most serious problems were animal overcrowding and discomfort, compounded by the overwhelming time and manpower required to care for the calves under these conditions. Subsequent alterations to provide two box stalls per chamber have been discussed earlier in Section 2.2.1 of this chapter. Meanwhile, the steers were maintained in the feedlot on a ration of hay and pelleted Calf Developer[s].

On July 26, 18 of the best-eating steers were divided into two groups according to weight (light or heavy), and ranked from lightest to heaviest within each group. Then one steer from each group was allocated randomly, firstly to an exposure chamber and secondly to a stall. This procedure resulted in the chambers all having a similar total initial liveweight.

Routine sampling of the chamber air revealed that after four days, naturally-occurring background levels of NH_3 had risen to surprisingly high values. Some chambers reached a level of 50 ppm. This

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[s] United Feeds Ltd., 102 St. - Saskatchewan Drive, Edmonton, Alberta.

undesirable situation was alleviated significantly, although not eliminated, by simply adding wood shavings on top of the old bedding, rather than cleaning the pens out every two days as had been the previous practice. This procedure actually resulted in an initial decline of background NH_3 levels which later stabilized within an acceptable range (< 20 ppm). Additional benefits included reduced labor time along with cleaner and more comfortable calves. A forage harvester was relegated to expedite handling of the shavings (Figure 3).

During the adjustment period, the author was introduced* to proper blood-sampling techniques, and collected several samples from a few calves for practice. At the same time, this provided an opportunity to test the animal handling facilities.

The calves also were introduced to a coarse-ground concentrate mixture that came bagged for convenience of handling and storage. The composition and nutrient analysis of the complete ration, as stated by the manufacturer[5], are given in Table 7. Due to slight problems with bloat, approximately 2 lb of alfalfa-brome hay were supplemented at each feeding. After 11 consecutive days of adjustment in the box stalls, the steers were ready to begin the pre-exposure period of Replicate 1. By this time all calves were on once-a-day feeding and leaving grain in their troughs.

The second load of 23 male calves for Replicate 2 arrived on August 28, 1974. This was a uniformly-sized lot of Hereford and Hereford-cross calves, with an initial weight range of 310 to 424 lb and an average weight per animal of 371 lb. Following the preliminaries of tagging and vaccinating, as described for the first replicate, 18 of the calves were

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* Advised by Malcolm Wharton, Surgical-Medical Research Institute, University of Alberta, Edmonton, Alberta.

randomly allocated to stalls. Between replicates, the stalls had been mucked out, aired to dry and sprayed with a fly insecticide before being re-bedded.

The coarse-ground ration (Table 7) was started at 1.5% of body weight divided into two feeds per day. The amount of grain was increased by 1/2 lb per animal per feeding until the calves were on full feed. Thereafter, this ration was supplied once-a-day so as to be available ad libitum. During the adjustment period, one slow-gaining steer was removed from a chamber and substituted with a calf that had been maintained in the feedlot expressly for replacement purposes. The replacement calf weighed within 10 lb of the cull. Following 18 days of adjustment, all calves were on full feed and prepared to enter the pre-exposure period of Replicate 2.

TABLE 7. COMPOSITION OF CALF RATION.

Ingredient	lb/ton
Oats	900
Barley	900
Beet Pulp	100
Sweet cattle supplement*	100

Nutrient:	Protein	Fat	Fibre	Ca	P
% :	12.5	4.6	8.5	0.56	0.50

* Includes 5 lb trace mineral salt and 0.1 lb Vit. A and D per 100 lb of supplement.

4.2 Exposure Periods.

Replicate 1 began at noon on Tuesday, August 6, 1974, with the 7-day pre-exposure period. Seven days of gas exposure followed, after which the calves were observed for a post-exposure period of another 7 days. In conforming to the management schedule, an experimental day was treated as extending between noon times of successive days. Tuesday, September 17, 1974, marked the start of the second replicate, similarly consisting of three consecutive one-week test periods.

The chamber lights were switched on at the beginning of each morning and shut off after the final evening check. The calves were removed from their stalls only for data collection procedures in the weigh room. Normally, this occurred three times each period and required at most 5 min. per calf. Fresh bedding was added at these times. Accordingly, during the gas exposure periods, the gases were infused continuously, except when interrupted for the data collection intervals.

5. Measurement of Response Criteria.

5.1 Feed and Water Consumption.

The manger facilities, conveniently accessible from outside the chambers, provided each calf with two removable feed boxes and two 2 1/2 gallon water pails (Figure 17). During the gas-exposure periods, this arrangement enabled feeding without shutting off the gas supply.

Feed was distributed from a cart (Figure 2) equipped with two spring scales graduated in tenths of a pound[t]. Consumption was recorded daily, just before noon, when leftover grain was weighed back and a fresh ration weighed in. In addition, a 2-lb flake of hay was provided in the

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[t] Hanson Dairy Scale, Model 600, Dial 0-30 lb, 60 lb capacity.

Figure 17. Box-stall feeding and water facilities.

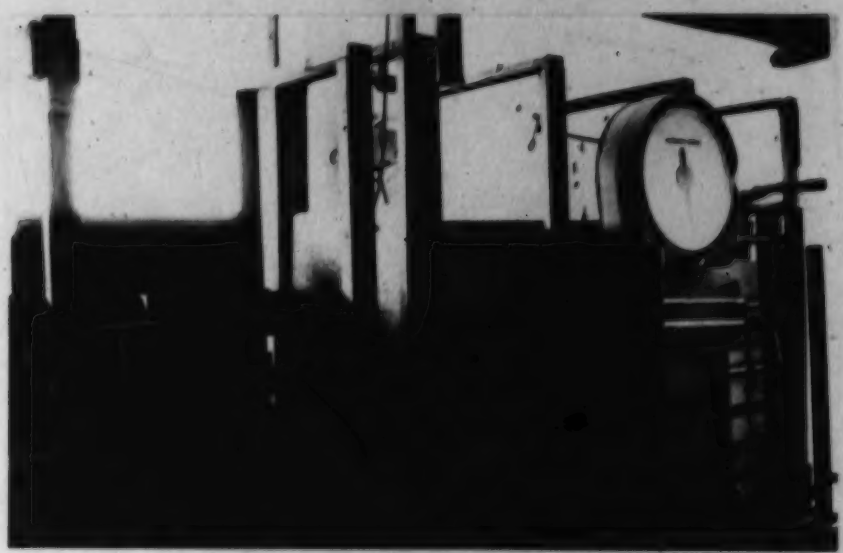

Figure 18. Stock scale and squeeze in weigh room.

second manger.

Drinking water from a pressure hose was added to each pail via a clear plastic tube mounted on the chamber wall and connected to a spigot at the bottom of the pail. A simple U-tube manometer setup permitted the volume of water in the pail to be read by comparing the water level in the tube to an underlying gauge board. Water intake was recorded, and the pails refilled, at 0800 hr and again late in the afternoon to ensure an ample supply throughout the night. Feed boxes and water pails were cleaned out as necessary.

5.2 Liveweight Gain.

The calves were weighed three times per period on the mornings of days 2, 4 and 7. Since each replicate only extended over a total of three weeks, feed and water were not withheld prior to weigh-in. The stock scale was furnished with a suspended platform and a dynamically-balanced weighbeam, and featured a pillar dial graduated in two-pound increments[u] (Figure 18). The scale was checked for zero adjustment before each calf was weighed.

5.3 Respiratory Rate.

Respiratory rates were taken each morning, just after switching on the chamber lights. During the gas- and post-exposure periods, rates were recorded a second time each evening. At these times, the calves were usually in a recumbent state and hence easier to observe.

Using a stop-watch, flank movements were counted for 15 or 30 second intervals when the respiration pattern was as steady as possible. Normally, two or more countings were averaged to determine the rate

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[u] Colt Industries, Fairbanks Morse Weighing Systems Division, 711 East St. Johnsbury Road, St. Johnsbury, Vermont 05819.

per minute. These proceedings were followed by evaluations of qualitative responses such as demeanor and evidence of sneezing, coughing and excessive lacrimation.

5.4 Rectal Temperature.

Rectal temperatures were measured on the mornings of days 2, 4 and 7 of each period when the calves were in the stock squeeze. Readings were taken with a battery-operated probe (Figure 19) and recorded to the nearest tenth of a degree Celsius. The instrument has an accuracy of ±0.15°C and a readability of ± 0.05°C[V], and was checked for meter adjustment before each set of recordings. To decrease the response time of the meter, the probe was placed in a container of warm tap water between insertions.

5.5 Blood Constituents.

5.5.1 Sampling Procedures.

Whenever possible, sampling days were scheduled so that blood was collected immediately after each calf's weight and rectal temperature had been recorded. In all, these proceedings took from two to five minutes per head, depending on the blood samples required. To effectively position the animals, each calf was restrained in the stock squeeze with a headgate, then haltered and snubbed around to a post (Figure 20). The external jugular vein was raised for puncture by using a throat latch and a wedge pressed into the jugular groove. The vessel was penetrated with a 20-gauge x 1 in. disposable needle attached to either a syringe or a Venoject tube holder[W], depending on the particular sample. The materials used for securing blood are illustrated in Figure 19.

[V] Tele-thermometer, Model 46 TUC, Yellow Springs Instrument Co., Inc., Yellow Springs, Ohio.

[W] Standard Hospital Supply Ltd., 15819 - Stony Plain Road, Edmonton, Alberta.

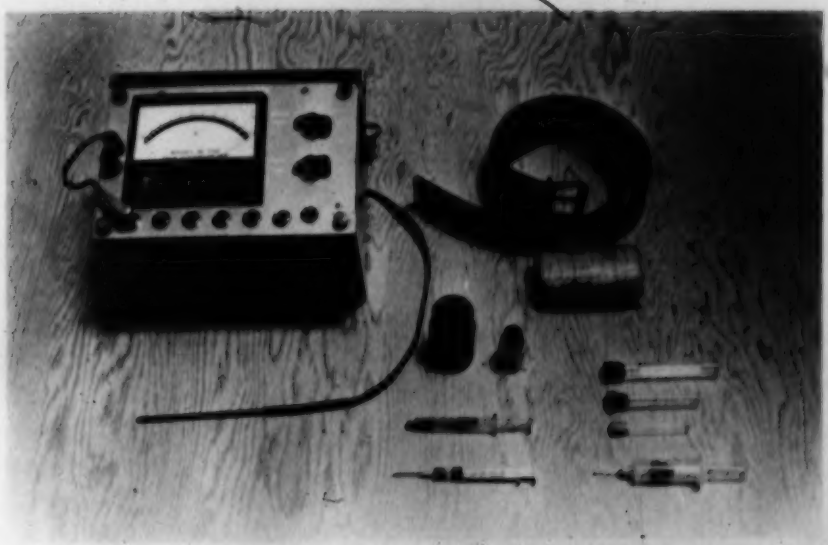

Figure 19. Temperature recorder with rectal probe (left) and blood sampling equipment (right) - throat latch assembly (top), syringes and accessories (bottom left), Venoject tubes and holder (bottom right).

Figure 20. Calf positioned in squeeze for blood collection purposes.

The Venoject tubes are supplied either plain with a silicone coating or with a measured amount of anticoagulant, and sufficient vacuum to draw the predetermined volume of blood. A double-pointed needle screws into the holder and a tube is placed so that the rubber stopper sealing the tube just rests on the short end of the needle. After the vein is punctured, the tube is pushed all the way into the holder, breaking the vacuum and causing blood flow into the tube. After the flow ceases, the tube may be removed and another tube inserted into the holder. This method permitted all Venoject samples to be collected from a single puncture. Table 8 lists the tubes used for samples tested during the experiment.

TABLE 8. VENOJECT BLOOD SAMPLES

Test	Tube Specifications	
	Draw (ml)	Anticoagulant
Complete Blood Count (C.B.C.)	3	EDTA (K3)*
Ammonia (NH_3)	5	Sodium heparin
SMA-12	10	none
Sulfhemoglobin (SHb)	5	Sodium heparin
B.U.N., Bili., S.G.O.T., L.D.H.**	5	Sodium heparin

* Ethylenediamine tetra-acetic acid (potassium salt)

** Blood urea nitrogen, bilirubin (total), serum glutamic-oxalacetic transaminase, lactic dehydrogenase, respectively.

Samples for blood gas (pO_2, pCO_2)*** and pH determinations were collected in either 3 ml plastic (disposable), or 2 ml glass syringes previously lubricated and sealed with mineral oil and wetted with sodium

*** p denotes partial pressure of gas.

heparin. The syringes were filled to capacity, capped immediately upon withdrawal, and stored in crushed ice.

All samples were labelled and preserved in a covered styrofoam cooler. Special care was taken to keep the bilirubin samples out of the light, while the blood ammonia tubes were kept in ice.

A series of samples for each test were taken at varying intervals to give a dynamic picture of blood changes occurring across the periods. Table 9 outlines the sampling schedule for both replicates.

TABLE 9. BLOOD SAMPLING SCHEDULE.

Replicate	Test	Exposure Period and Day																		
		Pre							Gas							Post				
		0	1	2	3	4	5	6	7	1	2	3	4	5	6	7	1	2	3	4	5	6	7
1	SMA-12	x									x				x								x
	SHb			x							x				x								x
	CBC	x									x				x								x
	NH_3	x									x				x								x
2	BUN, Bili, SGOT, LDH			x							x				x								x
	NH_3			x							x				x								x
	pO_2, pCO_2, pH							x							x								x

5.5.2 Laboratory Tests.

Immediately after sampling, the vials of blood were transported to Edmonton for analysis. Two laboratories were employed in this regard:

1) Surgical-Medical Research Institute (S.M.R.I.), University of Alberta, and

2) Dr. S. Hanson and Associates, Medical Laboratory (Hanson's Lab.), 203 Professional Building, 10830 - Jasper Avenue.

5.5.2.1 Hanson's Laboratory.

Hanson's Lab. conducted two different tests on samples taken during Replicate 1 only.

1) SMA-12.

This battery of tests was performed by continuous flow analysis on a Technicon Corporation SMA 12/60 Auto-Analyser system[x]. A serum chemistry graph of 12 test profiles is produced as per Instruction Manual T67-109-A. The following blood constituents were tested: calcium, inorganic phosphate, glucose, blood urea nitrogen, uric acid, cholesterol, total protein, albumin, total bilirubin, alkaline phosphatase, L.D.H., and S.G.O.T.

Since the SMA-12 samples were centrifuged at S.M.R.I. before the serum was taken to Hanson's Lab. for analysis, the delivery time from Ellerslie was approximately 1 1/2 hr.

2) Sulfhemoglobin.

Whole blood samples were analysed for sulfhemoglobin using the method of G.E. Cartwright, Diagnostic Laboratory Hematology: 316. Publ. Grune and Stratton, 757 - 3rd Ave., New York, N.Y. 10017.

5.5.2.2 Surgical-Medical Research Institute.

Samples were collected for the following tests:

1) Complete Blood Count.

The C.B.C., taken during Replicate 1 only, was conducted in accordance with standard hematological procedures[y]. The test included

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x Technicon Corporation, Tarrytown, New York, 10591.

y Personal communication, Supervisor of Biochemistry, S.M.R.I., University of Alberta, Edmonton, Alberta.

hemoglobin, hematocrit, white cell count, differential count and red cell count.

2) Blood Ammonia.

Blood NH_3 values for both replicates were determined from whole blood by the method of Natelson: Microtechniques of Chemical Chemistry: 96-101[y].

3) B.U.N., Bili., S.G.O.T., L.D.H.

These constituents were analysed during Replicate 2 using standard colorimetric and enzymatic methods[y] on blood serum.

4) pO_2, pCO_2, pH.

These measurements were performed on whole blood samples, taken during Replicate 2 only, using a Radiometer blood gas machine pH meter 27[z].

The delivery time for all samples transported between Ellerslie and S.M.R.I. was approximately 1/2 hr. However, since up to 1 1/2 hr could elapse between the times that the first and last calves were sampled, a question arose concerning the possibility of blood chemistry values changing during this interval. Subsequently, a feedlot calf was bled immediately before and after blood had been collected from the experimental calves, to provide samples for all the tests performed during the second replication. A comparison of the 'before and after' laboratory results indicated that, at least for the purposes of this study, the changes occurring in blood values between the two sampling times were insignificant. The series of blood chemistry reports for these samples are given in Appendix 2.

A further procedural check involved several vials drawn

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[z] Radiometer, Copenhagen, Denmark.

concurrently from the same calf and submitted for identical tests, while another trial was conducted to compare the suitability of plastic versus glass syringes for collecting blood gas samples. The respective outcomes confirmed that the sampling procedures produced consistent results, and that plastic syringes were equal to glass in preserving blood gas samples (Appendix 2).

5.6 Clinical Examination.

To evaluate possible qualitative effects of the gas exposures, a series of four clinical examinations of the experimental calves were conducted during Replicate 2 by a veterinarian*, at the author's request. The clinical and gross pathologic examinations, made while the calves were restrained in the stock squeeze, comprised the following sequence.

1) Pre-exposure evaluation.
2) Day 2 of gas-exposure period.
3) Day 6 of gas-exposure period.
4) Day 6 of post-exposure period.

6. Statistical Analysis.

Response data were subjected to a multiway analysis of variance. Sources of variation for all measurements except certain blood parameters consisted of ammonia (n = 3), hydrogen sulfide (n = 3), replications (n = 2) and calves (n = 2). Analyses were computed both across periods (n = 3) and within periods. The number of observations (days or samples) within each period varied according to response criteria and have been described in preceding sections of this chapter. Certain blood

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* Dr. B.E. Beck, Animal Disease Section, Veterinary Services Branch, Alberta Department of Agriculture, O.S. Longman Laboratory Building, Edmonton, Alberta.

constituents were tested during one replication only.

The sources of variation used as measures of error are shown in the Analysis of Variance Tables presented in the Appendix and were derived using the procedure for expected mean squares outlined by Hicks (61).

Analyses were computed using a Computing Services Analysis of Variance Program (127). The same program was employed to calculate the means and standard deviations of the environmental data, i.e., temperature measurements, relative humidities and gas levels.

V. RESULTS AND DISCUSSION

1. Environmental Conditions.

1.1 Background Gas Levels.

As mentioned previously, naturally occurring levels of NH_3 were encountered during the preliminary trials and persisted throughout the experiment, although somewhat under control. The increasingly deep layer of bedding presumably provided the manure pack with sufficient capacity to absorb and retain the volatile urine such that stable NH_3 levels were maintained. The means and standard deviations of NH_3 background measurements, for all chambers combined, were 12.9±5.5 ppm and 13.0±4.5 ppm during the pre- and post- exposure periods respectively.

Reference to the literature indicates that these values may not have been abnormally high under the circumstances. An investigation by the Swedish Institute of Agricultural Engineering showed that NH_3 occurred in practically all cattle and pig facilities surveyed (97). The highest contents, approximately 30 ppm, were measured in stalls with solid manure handling. A subsequent report (115) summarizing the results of the Swedish study concluded that concentrations of NH_3 from solid cattle manure have sometimes been troublesome if too little bedding material was used or there were no special drainage arrangements for urine. In this experiment, the tightly-sealed floors of the animal chambers prevented the urine from escaping. Laboratory column studies have shown that 25 to 90% of the nitrogen in cattle urine can be volatilized into the air as NH_3, and that about half the total nitrogen in cattle excrement is in the urine (92).

Comparable situations involving background NH_3 levels have occurred in other air-factor studies. During one experiment (42), the mean NH_3 concentration in an environmental chamber housing six or fewer weaner

pigs for controls was 8.0 ppm. In this case, the fecal material collected below a steel mesh floor and was emptied daily.

Consistent with the Swedish reports (97,115), no measurable amounts of H_2S were found to have been released from the manure pack. On the other hand, CO_2 occurred in all chambers because of the fact that this gas stems mainly from air exhaled by the animals (97). The mean and standard deviation of the CO_2 levels, averaged over the three exposure periods, were 0.1350%(1350 ppm) $\pm$ 0.0208%. A slight but consistent increase in the mean concentrations from 0.1273% to 0.1302% to 0.1476% for the pre-, gas- and post-exposure periods respectively, would suggest that the CO_2 content was sensitive to the liveweight gain of the calves. Levels in all chambers were nearly equal and showed similar trends as the trial progressed. Concentrations of CO_2 between 500 and 3000 ppm have been reported as normal in cattle facilities (55,97).

1.2 Infusion Gas Levels.

The actual concentrations of NH_3 and H_2S administered to the animal chambers during the gas-exposure periods were close to the desired levels (Table 10). Based on the NH_3 background levels of chambers to which NH_3 was not added, flow rates were adjusted to maintain the relative concentration difference of about 50 ppm between the low and 'zero' levels of NH_3, as originally planned. Accordingly, the low NH_3 levels were kept at approximately 65 ppm rather than 50 ppm. Increasing the high NH_3 levels proportionately was considered unnecessary since the discrepancy between 150 ppm and the low and 'zero' levels appeared sufficiently large.

Greater daily fluctuations at the high levels of H_2S than at the low levels, as represented by the standard deviations (Table 10), reflect the previously mentioned instability of the flowmeter floats as they were

progressively corroded by the gas. The comparably large standard deviation (± 14.6) for one high NH_3 level was also probably due to a flowmeter that had been used for high H_2S flow control during Replicate 1.

TABLE 10: AMMONIA AND HYDROGEN SULFIDE LEVELS DURING THE GAS-EXPOSURE PERIODS.

Treatment H_2S	Treatment NH_3	H_2S (ppm) Mean	H_2S (ppm) S.D.*	NH_3 (ppm) Mean	NH_3 (ppm) S.D.
0	0	0	-	18.4	±6.3
0	1	0	-	67.1	±7.5
0	2	0	-	157.1	±14.6
1	0	19.9	±2.1	15.8	±6.8
1	1	20.9	±3.1	66.7	±4.9
1	2	20.1	±3.0	153.7	±6.0
2	0	148.5	±7.8	13.4	±4.4
2	1	143.6	±12.7	65.5	±5.0
2	2	148.5	±12.1	154.4	±7.1

* Standard deviation.

1.3 Temperature and Relative Humidity.

The dry-bulb temperatures and relative humidities in the chambers during the exposure periods were similar (Table 11). The mean chamber temperature over the complete experiment was 69°F while the relative humidity averaged 63%. Mean daily chamber temperatures ranged between 61 and 76°F and the relative humidities between 53 and 75%. For any one day, the variation between individual chamber measurements was never greater than 5°F and 28% relative humidity.

TABLE 11: CHAMBER DRY-BULB TEMPERATURES AND RELATIVE HUMIDITIES DURING THE EXPOSURE PERIODS.

Measurement	Pre	Gas	Post
Temperature (°F)	70.2±3.2*	67.5±6.3	69.3±3.6
Relative Humidity (%)	62.1±4.3	65.5±5.9	62.4±6.9

* Data expressed as mean ± standard deviation.

2. Clinical Observations.

Clinical symptoms observed in the calves were characteristic of responses reported in the literature for other farm animals. From outward appearances, calf reactions to H_2S and NH_3 were similar in nature, but varied in severity according to treatment levels and combinations. Although to a limited extent, calves within a treatment sometimes exhibited varying effects, emphasizing that the clinical signs from exposure to toxic gases cannot be categorized rigidly.

The most prominent single observation during all treatments was eye irritation, evidenced initially by redness and excessive lacrimation. This appeared to be the principal effect of NH_3. Even slight but persistent watering of the eyes was detected for one control calf exposed to background levels while at infusion concentrations, NH_3 usually induced lacrimation and inflammation of the mucous membranes within the first few days of gassing. Additional symptoms included serous nasal discharge in amounts from slight at 65 ppm to profuse at 150 ppm, and infrequent dry coughing. Calves exposed to the high NH_3 level tended to keep their eyes closed, and periodically displayed an irregular respiratory pattern accompanied by

shallow breathing. Rubbing the head and eyes were interpreted as signs of irritation or discomfort.

The gross pathological examination revealed that the worst effect of NH_3 apparently was sufficient irritation to cause a slight inflammatory reaction in the cornea, evidenced by the migration and infiltration of leukocytes. Very slight cloudiness resulted. However, as exposure time progressed, the animals appeared to accomodate or adapt, suggesting that their tissue defence mechanisms had overcome the irritating effects of NH_3. Hence, the clinical symptoms were no longer readily apparent by the latter part of the exposure period.

At all levels of H_2S, definite detrimental effects were apparent. At times the calves appeared lethargic and stood with heads lowered, eyes closed (Figure 21), tongues out and slobbering, while at other times, discomfort and distress were evidenced by head shaking and rubbing, blowing through and licking the nose, pawing, tail switching, restlessness, and uneasiness. Scouring and vomiting were observed in isolated cases. This overt behavior indicated irritant and possible neural effects of H_2S in the calves. Respiratory patterns often were erratic and varied from shallow breathing and panting to breath holding and labored breathing or dyspnea. Spasmodic coughing also was noted. Generally, the above conditions appeared to have lessened by the last one or two days of exposure.

Rather than producing a primary irritant effect as did NH_3, H_2S appeared to have a direct degenerating effect through cytotoxic mechanisms on the viability of the exposed membranes of the eye - the conjunctiva and cornea, and of the nasal mucous membranes. At both levels of H_2S, signs of photophobia were evidenced by strabismus and refusal to open the eyes. These responses were particularly apparent when the chamber lights were

switched on after the calves had been in darkness overnight, and when the animals were exposed to daylight in the weigh and handling room (Figure 21). At the higher levels, corneal opacity was severe (Figure 22). In two notable cases, total corneal opacity restricted vision to the extent of apparent blindness; the calves ran past doors and bumped into walls and other objects. The examining veterinarian (15) concluded that exposure to 20 ppm H_2S for one week resulted in tissue damage to the cornea which could be considered permanent in nature. Even though the worst of these lesions would probably heal to satisfactory functional capabilities, there should be some residual damage. At 150 ppm H_2S, sufficient damage was present to weaken the cornea and cause keratoconus. This would suggest that rupture of the organ is a very real possibility where animals might be exposed to such levels for periods of one week or more. The eyes of white-faced calves appeared to be the most susceptible to inflammation - an effect that probably is related to the light skin pigmentation.

Irritation of the mucous membranes was emphasized by inflammation, congestion, nasal discharge (Figure 21) and hemorrhage or epistaxis of the nasal membranes. The insidious action of H_2S at 150 ppm, alone and in combination with NH_3, induced varying degrees of epistaxis in five out of the six calves examined during the second replication. Pronounced effects were observed for the 150 ppm H_2S - 150 ppm NH_3 treatment. During Replicate 1, blood flowed from the nostrils of one of the two calves exposed to this treatment after 21 hours. In addition, as diagnosed by the presiding veterinarian*, rumen function was decreased in frequency and intensity of movement. The animal was treated with antibiotics and an estrogenic blood

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* R.H. Shute, D.V.M., Leduc Veterinary Hospital, Leduc, Alberta.

Figure 21. Clinical symptoms of calves exposed to 150 ppm H_2S.
Top: Lethargic appearance, eyes closed to chamber light and lacrimating excessively. Bottom: Serous nasal discharge, excessive lacrimation and signs of photophobia evidenced by strabismus.

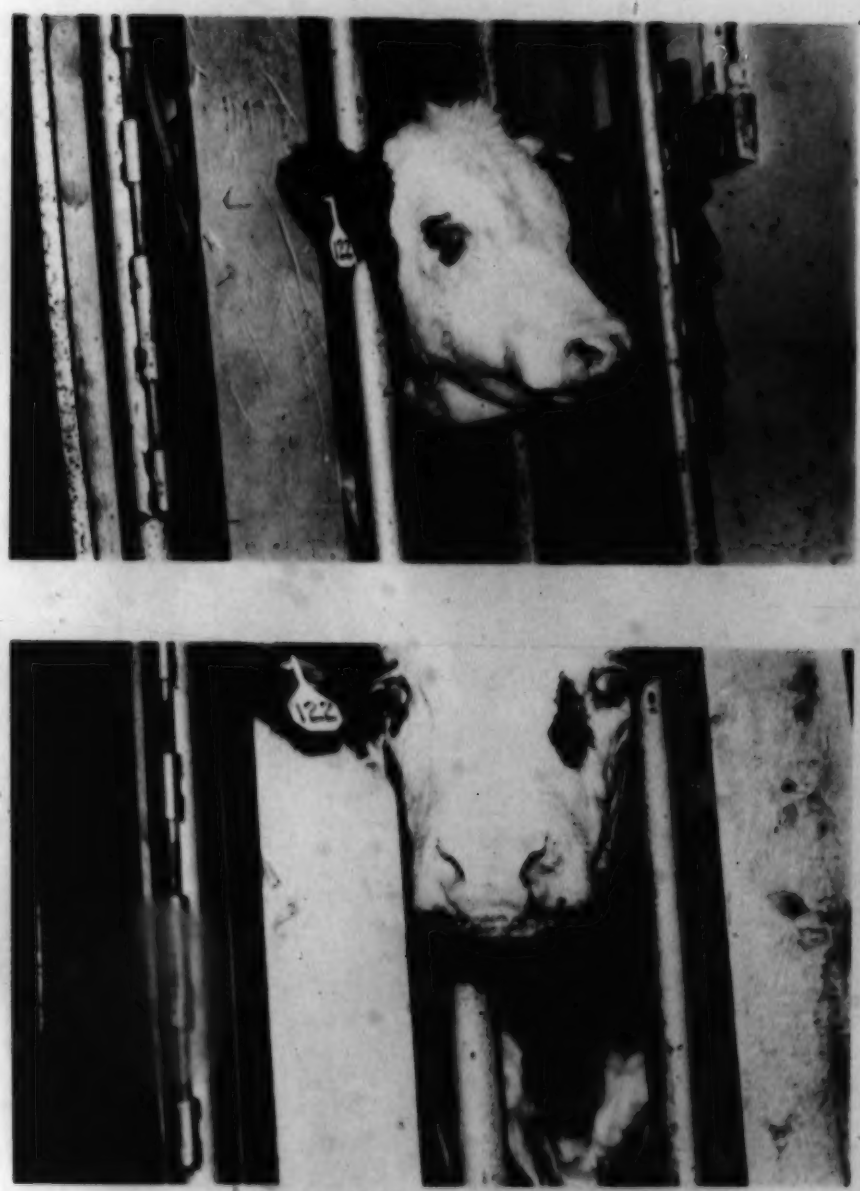

Figure 22. Clinical symptoms of calves subjected to 150 ppm H_2S and 150 ppm NH_3 in combination. Excessive lacrimation, corneal opacity of the eye, nasal discharge and epistaxis.

clotting agent and turned out. Three days later, and apparently fully recovered, the steer was returned to the test chamber on the premise that a repeat reaction would confirm that gas exposure was the causative agent. Within 23 hours, a bloody nasal discharge was again observed. In accord, both calves exposed to the same treatment in Replicate 2 exhibited severe epistaxis (Figure 22).

A general tendency to hemorrhage has been reported in alleged chronic manure gas poisonings of cattle (67). Even in cows without manifest illness, the blood coagulation time was prolonged (16,109). Sallvik (109) noted that the coagulation was normalized within a few hours after stopping agitation of liquid manure which had produced about 1 ppm H_2S in the animal area.

In conclusion, although the effects of H_2S were definitely more severe and extensive than those of NH_3, the clinical observations tended to obscure the effects of H_2S and NH_3 in combination. Eye lesions were more consistently severe for H_2S alone, suggesting that NH_3 did not intensify the action of H_2S, and might even have a sparing effect. However, as previously discussed, the severe reactions of the nasal mucous membranes to the high level mixture of H_2S and NH_3 would suggest an additive or synergistic interaction. There appears to be no obvious explanation for these differences.

At the post-exposure examination, conducted six days after gas infusion ceased, clinical symptoms were still apparent in the more severe cases. However, affected areas generally showed signs of healing, and no after effects were observed. Details of the four clinical and gross pathologic examinations of the calves are included in Appendix 3.

3. Production and Performance Data.

3.1 Feed and Water Consumption.

Feed.

Data on the effects of NH_3 and H_2S on feed consumption are given in Table 12. Figure 23 presents the average daily feed consumptions for the pre (control)-, gas- and post-exposure periods. Ammonia alone at any treatment level did not have an appreciable effect on mean period consumptions. The natural background NH_3 concentrations apparently did not adversely affect appetites of the control (OH_2S - ONH_3) calves over the three-week trial, as illustrated by the similar mean period values. Of course, the possibility exists that appetites of all calves were suppressed by the background NH_3 even before the test periods began, i.e. during the adjustment period, thereby misrepresenting the pre-exposure intakes as being normal. However, if such were the case, consumption of the control animals would be expected to decline as time progressed. Actually, the maximum mean period intake of the controls was recorded for the second week of exposure when the background NH_3 concentration averaged 18 ppm - approximately 3 ppm higher than either of the other periods. This argument is demonstrated more emphatically by the 65 ppm NH_3 treatment in which consumption during the gas-exposure period increased 9% above the pre-exposure mean, and then fell back slightly in the post-exposure period.

Even though the high level of NH_3 alone did not reduce the mean gas-period intake any more than 0.6%, consumption dropped by 16% on day 1 of infusion before returning to normal the next day. This initial decline, along with the abrupt increase in feed consumed after the gas was turned off, suggests that 150 ppm NH_3 may have suppressed appetites.

Hydrogen sulfide alone at both low and high levels reduced

TABLE 12: MEANS OF DAILY FEED CONSUMPTION (POUNDS/CALF).

Treatment H_2S	Treatment NH_3	Period Pre	Period Gas	Period Post
0	0	13.58	13.95	13.59
0	1	12.48	13.59	13.41
0	2	11.75	11.68	13.53
1	0	12.08	11.68	12.86
1	1	12.76	12.41	13.48
1	2	12.59	11.76	11.32
2	0	12.78	9.49	14.26
2	1	13.45	10.81	13.61
2	2	11.96	8.14	13.53
		S.E.M.+ = 0.85		
0		12.60	13.07	13.51
1		12.48	11.95	12.55
2		12.73	9.48	13.80
		S.E.M. = 0.49		
	0	12.81	11.71	13.57
	1	12.90	12.27	13.50
	2	12.10	10.53	12.79
		S.E.M. = 0.49		
		12.60	11.50	13.29
		S.E.M. = 0.28		

Treatment H_2S	Treatment NH_3	Day of Period 1	2	3	4	5	6	7
		Gas Exposure						
0	0	13.95	14.68	13.60	13.95	14.08	13.58	13.82
0	1	13.78	14.52	13.75	12.88	13.08	13.08	14.02
0	2	9.90	11.70	11.60	12.38	12.08	11.62	12.50
1	0	11.70	10.42	10.42	10.65	11.98	13.20	13.38
1	1	13.08	11.95	12.85	11.90	12.38	11.92	12.82
1	2	13.35	11.30	12.35	12.20	11.60	11.28	10.28
2	0	11.20	8.45	7.60	8.48	11.30	9.40	10.02
2	1	11.22	9.78	10.65	11.68	11.58	10.22	10.65
2	2	8.20	6.00	8.00	7.60	8.00	9.60	9.58
		Post Exposure						
0	0	15.05	15.45	14.52	14.22	13.08	13.02	9.75
0	1	14.08	11.58	12.62	13.68	13.42	14.35	14.18
0	2	14.08	13.40	11.95	13.20	13.08	14.20	14.82
1	0	13.40	12.08	10.52	13.22	12.95	14.05	13.82
1	1	12.88	13.68	13.12	13.58	14.15	13.98	12.98
1	2	10.38	9.92	10.32	10.27	12.00	12.82	13.50
2	0	12.80	12.88	14.62	13.70	14.60	15.50	15.75
2	1	13.25	13.68	14.62	12.78	12.48	14.20	14.30
2	2	12.38	11.38	14.05	13.60	14.48	15.02	13.82
		S.E.M. = 0.14						

+ Standard error of mean

average intakes during the gas period by 3.5 and 26% respectively, compared to the pre-gas means. Exposure to 20 ppm H_2S reduced intake by as much as 14% on day 2 and normal consumption was not regained until day 5. The pre-exposure mean intake was surpassed on days 6 and 7, suggesting that 20 ppm H_2S may only temporarily induce anorexia. Intakes for the post-exposure period remained at about the same level as that for the last two days of gassing. At 150 ppm H_2S, consumption decreased by 12.5% on day 1 and appetites were affected adversely throughout the remainder of the gas period. Intake dropped a maximum of 40.5% on day 3 and was still 22% below normal on day 7. Food consumption recovered during the first day of post exposure and increased thereafter to result in a mean period intake 11.5% above that of the pre-exposure norm.

Consideration of the H_2S and NH_3 mixtures shows that 20 ppm H_2S with 65 ppm NH_3 did not affect appetites any more severely than did 20 ppm H_2S alone. Daily intake was not reduced until day 2 of gas exposure and was never more than 7% below normal. The gas period mean intake was only 2.5% below that of the control period. These results suggest that 65 ppm NH_3, rather than having a synergistic (intensifying) effect with 20 ppm H_2S, may have either a simple additive (nil interaction) or even an antagonistic (sparing) effect.

The combination of 20 ppm H_2S and 150 ppm NH_3 caused the mean gas-period feed consumption to decrease by 6.5%. Since this is approximately twice the percentage reduction than that for 20 ppm H_2S alone, the high NH_3 level may add to or possibly potentiate the action of H_2S. As such, the earlier suggestion that NH_3 in combination with H_2S may have a sparing effect no longer appears valid. Intake dropped on day 2 of gassing and reached a low of 82% of the pre-exposure norm on day 7. Consumption did

not return to normal until six days later. Accordingly, this was the only treatment for which mean post-exposure intakes failed to exceed the control norm when a reduction in consumption had occurred during the gas period.

Data for the 150 ppm H_2S - 65 ppm NH_3 treatment reveal that feed intake declined an average of 19.5% during the gas period. This reduction is not as great as that for 150 ppm H_2S alone, which concurs with the previous suggestion that 65 ppm NH_3 does not appear to intensify the effects of H_2S. The trend in loss of appetite was similar to that for the high level of H_2S alone, except less severe. The lowest intake, recorded on day 2, was 73% of the control mean. Normal amounts of feed were consumed during the first day of post exposure.

The most drastic reduction in feed intake resulted from exposure to the high levels of NH_3 and H_2S together. Average consumption for the gas-exposure period was 32.5% below that of the control period. Inappetence was evident during the entire week, with intake falling 50% below normal by day 2. On the last two days, consumption recovered to 80% of normal. One day after gas flow ceased, the pre-exposure mean intake was surpassed. Again, in agreement with the 20 ppm H_2S - 150 ppm NH_3 results, indications are that NH_3, at the high concentration, may intensify the adverse effect of H_2S on feed consumption. At least, the effects of 150 ppm NH_3 and 150 ppm H_2S appear to be additive.

Analysis of variance (Appendix 4) showed that neither the main factors (H_2S and NH_3) nor the simple interaction of H_2S and NH_3 were significantly different. However, the difference in feed consumption between periods was highly significant ($P < 0.01$), as was the interaction between periods and H_2S levels. Figure 24 shows the nature of this interaction. In addition, the difference among days was significant

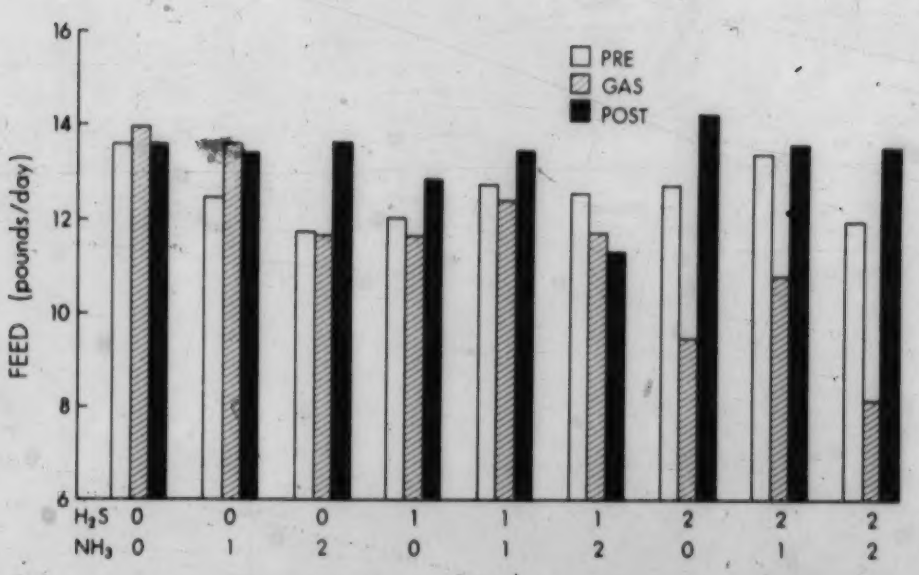

Figure 23. Period means of daily feed consumption per calf.

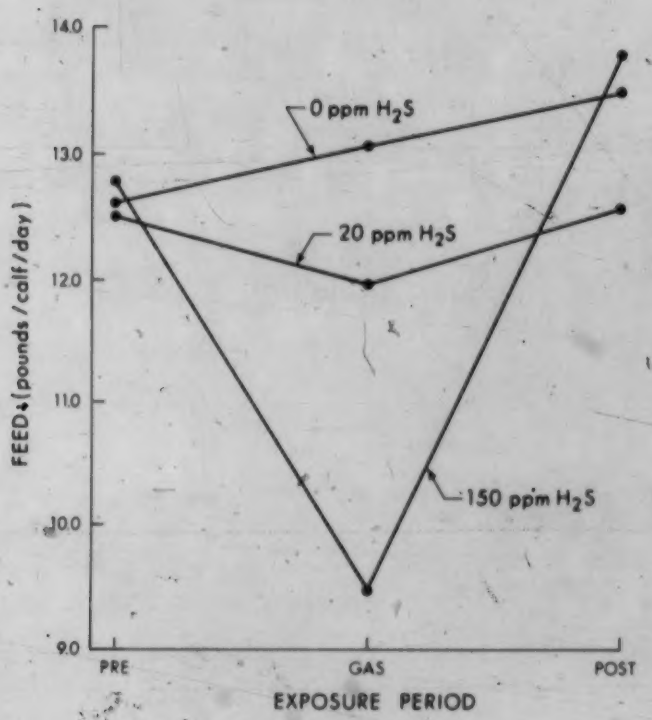

Figure 24. Interaction of exposure period and hydrogen sulfide concentration on feed consumption.

($P < 0.05$). For reasons presented in the preceding discussion and with particular reference to the response curves of Figure 24, the significant adverse effects on feed consumption can be attributed primarily to the 150 ppm H_2S factor level.

Water.

Means of daily water intake are presented in Table 13 and shown graphically for each period in Figure 25. As was the case with feed consumption, NH_3 alone at background, low or high levels apparently did not suppress water intake. In fact, consumption for all three treatments increased during the gas-exposure period, which might suggest a direct correlation between NH_3 concentration and greater degree of thirst. Seemingly though, no such relation can be ascribed, since the high NH_3 level caused the least increase in consumption. Furthermore, elevated post-exposure mean intakes of 5.5 and 13% above those at concentrations of 65 and 150 ppm respectively, would tend to refute the possibility that NH_3 enhances the desire to drink. Instead, the marginal increase during the 150 ppm NH_3 exposure compared to the relatively large rise for the post-exposure period indicates that the effect of NH_3, if any, is to inhibit water consumption.

The 20 ppm H_2S treatment showed a very slight consumption increase for the gas period, but at 150 ppm H_2S mean period intake was only 75.0% of normal. Water intake at the latter concentration declined 25.5% during the first day and then remained nearly constant throughout the gas period. When the H_2S infusion ended, consumption for the 20 ppm treatment again rose slightly, while the desire to drink recovered immediately for the calves exposed at 150 ppm, the average post-exposure consumption almost equalling that of the control period.

TABLE 13: MEANS OF DAILY WATER CONSUMPTION (GALLONS/CALF).

Treatment H_2S	Treatment NH_3	Period Pre	Period Gas	Period Post
0	0	3.60	3.71	3.52
0	1	3.56	3.77	3.95
0	2	3.00	3.08	3.49
1	0	3.13	3.19	3.25
1	1	3.86	3.45	3.69
1	2	3.72	3.47	3.68
2	0	3.47	2.60	3.50
2	1	3.90	3.42	4.23
2	2	3.24	2.10	3.24
		S.E.M. = 0.23		
0		3.38	3.52	3.65
1		3.57	3.37	3.54
2		3.54	2.71	3.66
		S.E.M. = 0.13		
	0	3.40	3.17	3.42
	1	3.77	3.54	3.96
	2	3.32	2.88	3.47
		S.E.M. = 0.13		
		3.50	3.20	3.62
		S.E.M. = 0.08		

Treatment H_2S	Treatment NH_3	Day of Period 1	2	3	4	5	6	7
				Gas Exposure				
0	0	3.40	3.78	3.62	3.92	3.88	4.22	3.12
0	1	3.82	3.78	4.12	3.85	3.72	3.80	3.30
0	2	2.82	2.75	3.08	3.60	2.95	3.55	2.80
1	0	3.40	3.20	3.42	2.92	3.22	3.35	2.80
1	1	3.28	3.25	3.42	3.60	3.65	3.78	3.15
1	2	3.12	3.50	3.32	3.75	3.60	3.65	3.32
2	0	2.65	2.92	2.68	2.35	2.82	2.52	2.28
2	1	3.15	3.20	3.48	3.92	3.52	3.75	2.90
2	2	1.82	1.95	1.98	2.35	1.92	2.32	2.32
				Post Exposure				
0	0	3.60	4.00	3.60	3.90	3.58	3.10	2.90
0	1	4.10	3.60	3.70	3.92	4.05	3.90	4.38
0	2	3.28	3.60	2.55	4.15	3.22	4.00	3.60
1	0	3.32	3.38	2.90	3.32	3.00	3.50	3.30
1	1	3.82	3.75	3.72	3.32	3.75	3.68	3.80
1	2	2.95	3.82	3.82	3.48	3.82	3.58	4.30
2	0	3.38	3.50	3.60	3.20	3.42	3.60	3.80
2	1	4.10	4.32	4.38	4.05	4.00	3.98	4.80
2	2	2.55	3.18	2.68	3.68	3.48	3.62	3.50
				S.E.M. = 0.04				

Both 20 ppm and 150 ppm H_2S in combination with 65 ppm NH_3 caused 12.8% declines in mean period consumptions. For the low level H_2S - NH_3 mixture, this indicates a far more severe curtailment of water intake than for either gas concentration alone, when in fact, intakes had increased slightly. Inconsistently, the 150 ppm H_2S - 65 ppm NH_3 treatment caused a less drastic reduction in consumption than did the high level of H_2S alone. Intakes for the low level combination rose to just below normal on day 1 of post-exposure and remained so during the subsequent days. In contrast, the calves subjected to 150 ppm H_2S and 65 ppm NH_3 consumed more water than normal during the first post-exposure day and continued likewise for the rest of the period, surpassing the pre-exposure mean by 7.5%.

The high level of NH_3 in combination with 20 ppm H_2S decreased mean intake by 5.5%, which is not as severe as the low level combination. However, since consumptions during the gas- and post-exposure periods for both of these treatments were similar, the adverse effects of the combinations probably are similar also. On the other hand, 150 ppm NH_3 appeared to intensify the effect of 150 ppm H_2S, with this treatment having the most drastic reduction in consumption. A sharp drop of 44% on day 1 of gas exposure tended to recover during the period to a maximum of 72% of normal on the last two days. The gas period intake averaged 34.5% below the control mean. Two post-exposure days passed before consumption attained the normal level, but the average period intake equalled that of the pre-exposure period.

The variation in response differed significantly (Appendix 4) for exposure periods ($P < 0.01$) and also for the interaction between H_2S levels and exposure periods ($P < 0.05$). This interaction is

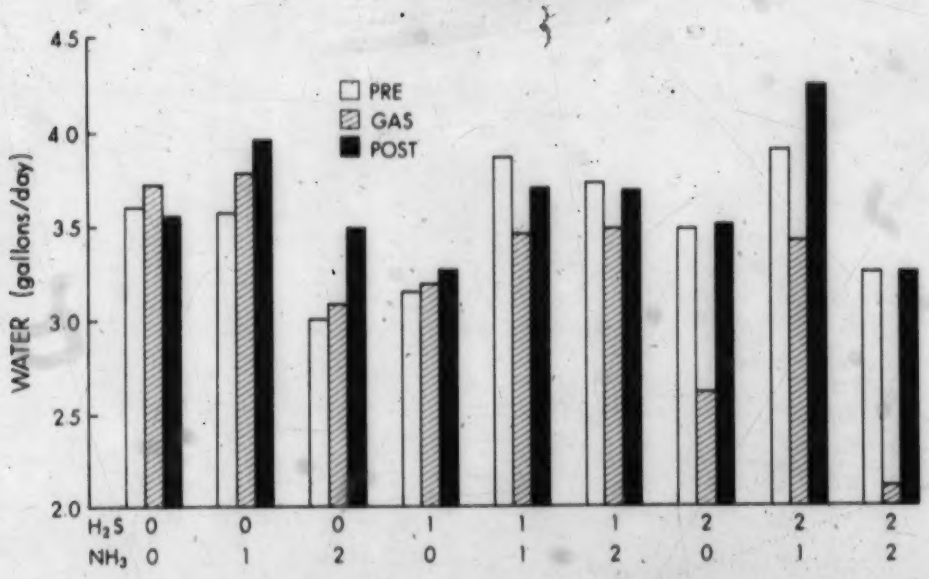

Figure 25. Period means of daily water consumption per calf.

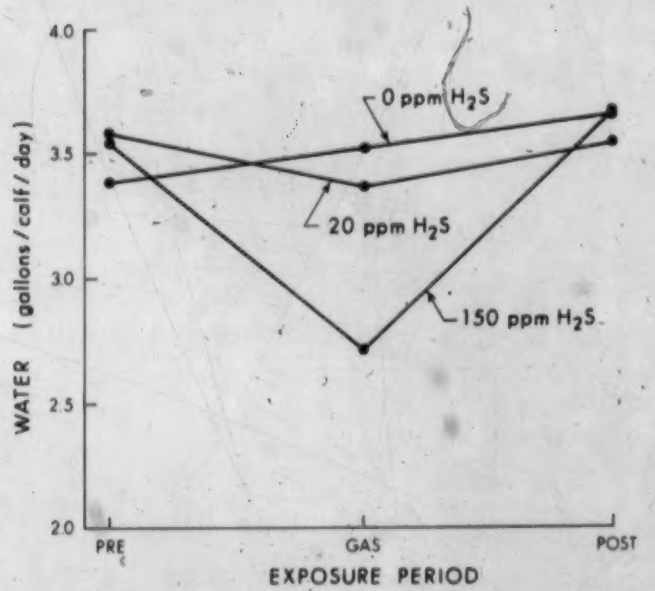

Figure 26. Interaction of exposure period and hydrogen sulfide concentration on water consumption.

illustrated in Figure 26. As with the trends in feed consumption, a negative response to the high H_2S level (150 ppm) accounts largely for the discrepancies in daily water intake between periods. A significant ($P < 0.05$) interaction between days and periods, similarly, can be attributed mainly to the 150 ppm H_2S level, notably in combination with 150 ppm NH_3.

Feed and Water.

Generally, NH_3 alone at background (15 ppm), low (65 ppm) or high (150 ppm) levels had little or no adverse effect on feed and water consumption. In contrast, H_2S alone reduced feed intake by amounts proportional to concentration, i.e. by 3.5 and 26% at 20 and 150 ppm H_2S respectively. The high level of H_2S decreased water consumption by the same percentage as feed, whereas the low H_2S level did not appear to affect water intake.

Data for the 65 ppm NH_3 - H_2S combinations indicated that appetites were reduced no more severely by the gas mixtures than when H_2S at either 20 or 150 ppm was infused alone. Water intake declined for both treatments. These results suggest that the effects of NH_3 and H_2S at these levels are additive; 65 ppm NH_3 did not increase the assault of H_2S on feed and water consumption. The lack of a potentiating interaction between H_2S and NH_3 appears to be supported by a report of Curtis et al (38) showing that 50 ppm NH_3 with 2 ppm H_2S had no more effect on pig performance than did H_2S alone at 8.5 ppm.

On the other hand, NH_3 at 150 ppm seemingly intensified the adverse effects of both 20 and 150 ppm H_2S on feed and water consumption. Although these results suggest a possible synergism between H_2S and the high level of NH_3, the lack of complete consistency

in response to the low and high NH_3 concentrations favors a repeat proposal that the effects of H_2S and NH_3 are additive.

For all treatments causing anorexia, the effects usually were apparent during the first day of gas exposure or shortly thereafter. With similar rapidity, in cases where feed consumption was suppressed throughout the infusion period, appetites generally recovered one or two days after the gas flow ceased. These trends in response also held true for water intake.

Field measurements conducted in cowsheds by Noren et al (97) found no H_2S, 5 to 10 ppm NH_3 and 1500 to 3000 ppm CO_2, as long as liquid manure remained undisturbed. Under these conditions, the desire of calves to consume food and water would not appear to be appreciably affected. However, other workers have suggested that practical levels originating within a cattle building can reach 20 ppm H_2S (120) and 30 ppm NH_3 (55). The results indicate that such gas concentrations could well be detrimental to the performance of calves. During mixing operations, gas concentrations at animal level have been reported to range from 15 to 600 ppm H_2S (55,68,97) and up to 700 ppm NH_3 (55). Barring death, a drastic and immediate reduction in consumption would be expected under these circumstances.

Although the mechanisms inhibiting the desire to consume food and water are not indisputably obvious, several possibilities exist. One of the most logical explanations is that the gases may have reduced the palatability of the feed and water in the mangers. This effect may be applicable particularly to the desire to drink, as both H_2S and NH_3 are soluble in water at room conditions. Reasons supporting this suggestion are that water intakes did not show a

tendency to recover over the gas period as did feed consumption. Furthermore, when the water pails were cleaned out following the last day of gas exposure, a distinct odor of H_2S could be detected. Presumably the more soluble NH_3 was also present in chambers that received NH_3 infusion, but since the odor threshold of man for NH_3 is higher than that for H_2S, its presence may have escaped detection. The supply of fresh water immediately after the gas-exposure period also may explain, at least partially, the rapid recovery of water consumption.

Pursuing the same subject, perhaps the restricted intake of water caused a decrease in feed consumption; an interaction which has been found with dry Holstein cows (112). Possibly the drive to consume food overcame the effect of water restriction during the latter days of gas exposure, thereby accounting for the trend to regain appetites. However, whether an immediate decrease in feed consumption could be due singularly to a synchronous decrease in water intake is doubtful.

Another possibility, as suggested by Hays (58), is that the reduced feed and water consumptions were due to the effect of sulfide on the taste receptors, or to decreased salivation. While H_2S clearly was the more detrimental factor influencing appetites, the apparent observed eagerness of calves exposed at even the high concentrations to consume fresh feed would tend to refute this proposal - at least with respect to feed.

Since H_2S has neurological effects of headache and gastrointestinal disturbances in man at concentrations below 150 ppm (91), the occurrence of similar symptoms could be understood for cattle. Hays (58) has reported that hypothalamic centers in the brain of goats have been shown to control feed and water intake, and hence neural

control of appetite and thirst could possibly be affected with exposure to H_2S. Because the mammalian body reportedly has the ability to rapidly oxidize H_2S in the blood (60), the prompt recoveries after the gas was turned off appears feasible.

3.2 Live-Weight Gains.

Since feed and water were not witheld prior to weigh-in, and the experiment was of relatively short-term, live-weight changes largely would be expected to reflect immediate differences in consumption and body fluid content. With this acknowledgement, average daily gains and feed conversion efficiencies cannot be considered as having absolute significance. However, the weight data (Table 14) indicate some interesting trends. As shown in Figure 27, NH_3 and the low H_2S treatments did not appear to appreciably affect weight gain, as evidenced by the similar gains for the gas-and post-exposure periods. The fact that some of the calves, notably those exposed to 150 ppm NH_3 and the NH_3 - 20 ppm H_2S combinations, reduced feed and water intakes during exposure may indicate a retention of body fluids due to infrequent urination.

In comparison, changes at the high level H_2S treatments would suggest that these exposures have an adverse effect on weight gains; the 150 ppm H_2S - 150 ppm NH_3 combination even caused an apparent loss of weight. Granted, this well may be due to decreased consumption and loss of body water through scouring. Nonetheless, the liveweight gains displayed during the post-exposure period are of relevance. These results would suggest firstly that exposure for up to one week at even the high gas levels may not have an extended detrimental effect on live-weight gain - the calves appeared to recover from any set-back within a few days. Such occasions may occur in practice when manure

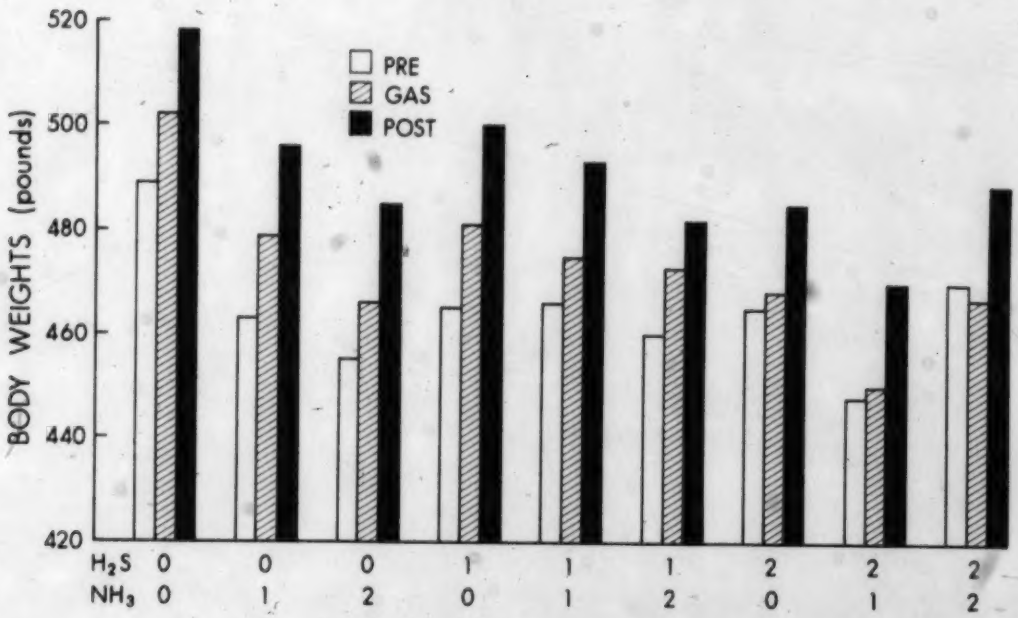

Figure 27. Period means of body weight per calf.

TABLE 14: MEANS OF BODY WEIGHTS. (POUNDS/CALF).

Treatment H_2S	Treatment NH_3	Period Pre	Period Gas	Period Post
0	0	489.4	502.9	518.9
0	1	463.0	479.6	496.2
0	2	455.8	466.8	485.6
1	0	465.8	481.2	500.2
1	1	466.2	475.1	493.0
1	2	460.1	473.1	482.4
2	0	465.9	468.4	485.5
2	1	448.8	450.6	470.3
2	2	470.3	467.7	488.9
		S.E.M. = 3.30		

Treatment H_2S	Pre	Gas	Post
0	469.4	483.1	500.2
1	464.0	476.5	491.9
2	461.7	462.2	481.6
		S.E.M. = 1.90	

Treatment NH_3	Pre	Gas	Post
0	473.7	484.2	501.5
1	459.3	468.4	486.5
2	462.0	469.2	485.7
		S.E.M. = 1.90	

Pre	Gas	Post
465.0	473.9	491.2
	S.E.M. = 1.10	

pits are cleaned out.

Secondly, however, if trends exhibited by calves at the high H_2S treatments were to be continued for weeks or months rather than days, indications are that weight gains would be severely curtailed. Although exposure to the elevated levels that induced this response may be unrealistic, equivalent effects would appear possible for prolonged exposures to the NH_3 - low H_2S levels since feed and water intakes were suppressed by these treatments.

4. Physiological Data.

4.1 Respiratory Rate.

The mean respiratory rates are presented in Table 15, while changes in the rates for each period are illustrated in Figure 28. Mean frequencies for the control calves tended to decline over the trial from a pre-exposure high of 85/min to a post-exposure low of 78/min. The decline probably was due more to a progressive adaptation to chamber conditions than to the effects of background NH_3. This contention, however, does not exclude the possibility that prolonged exposure to such low levels of NH_3 may tend to depress respiratory frequency. Ammonia alone at 65 ppm caused a 3/min increase in rate while at 150 ppm the rate decreased slightly by 1/min. These results indicate that NH_3 does not affect respiratory rate sufficiently to show definite responses. Nevertheless, the apparent increase at 65 ppm and decrease at 150 ppm suggests that low and high levels of NH_3 might affect the respiratory system differently.

As with NH_3, the changes caused by H_2S alone were fairly inconsistent and indecisive. At 20 ppm, H_2S induced a rise of

TABLE 15: MEANS OF RESPIRATORY RATES (PER MINUTE/CALF).

Treatment H_2S	Treatment NH_3	Period Pre	Period Gas	Period Post
0	0	85.2	84.0	77.8
0	1	81.1	84.3	95.6
0	2	75.8	75.1	80.9
1	0	72.2	74.8	74.9
1	1	79.2	82.2	79.5
1	2	76.3	76.4	73.6
2	0	82.4	78.8	86.8
2	1	79.1	79.5	88.1
2	2	86.9	62.8	84.9
		S.E.M. = 8.3		
0		80.7	81.1	84.8
1		75.9	77.8	76.0
2		82.8	73.7	86.6
		S.E.M. = 4.8		
	0	80.0	79.2	79.8
	1	79.8	82.0	87.8
	2	79.7	71.4	79.8
		S.E.M. = 4.8		
		79.8	77.6	82.5
		S.E.M. = 2.8		

Treatment H_2S	Treatment NH_3	Day of Period 1	2	3	4	5	6	7
				Gas Exposure				
0	0	83.0	92.5	82.5	87.8	79.8	97.0	65.2
0	1	98.2	81.5	93.0	80.8	74.8	88.0	74.0
0	2	79.0	75.0	84.5	81.5	74.8	69.2	62.0
1	0	75.5	76.0	80.0	72.2	66.8	85.0	68.2
1	1	80.5	83.5	89.2	88.2	84.5	82.0	67.8
1	2	78.0	85.0	67.2	84.5	78.5	76.5	65.0
2	0	82.5	79.5	87.0	80.5	82.8	77.0	62.2
2	1	85.0	83.5	77.5	84.8	80.2	79.0	66.5
2	2	77.5	67.2	64.2	64.2	53.0	60.8	52.5
				Post Exposure				
0	0	89.5	83.0	69.8	74.8	64.2	75.5	88.0
0	1	96.5	95.0	92.5	100.5	86.5	88.5	110.0
0	2	81.0	80.0	75.2	79.8	68.5	82.0	100.0
1	0	84.0	76.2	73.8	73.8	61.5	62.8	92.5
1	1	78.5	83.0	79.5	73.5	75.2	76.0	91.0
1	2	71.2	68.5	70.5	72.5	69.2	82.0	81.0
2	0	75.8	79.0	86.0	84.5	78.5	96.5	107.0
2	1	82.0	97.0	88.8	87.5	80.2	75.0	106.0
2	2	80.8	81.5	87.0	78.5	88.2	87.0	91.0
				S.E.M. = 6.8				

approximately 3/min whereas 150 ppm caused a decrease in rate of about 3/min. Like the NH_3 exposures, these responses suggest that H_2S conceivably may elicit different or even opposite effects at low and high concentrations. For both H_2S and NH_3 alone, frequencies during the post-exposure period averaged above normal at either low or high levels. This could be compatible with a general tendency for H_2S and NH_3 to suppress respiratory rate. The lowest rates for both H_2S and NH_3 alone at 150 ppm occurred on day 7 of the gas period. However, a similar trend was noted for the control calves, suggesting that although respiratory frequency was lowest during the high level exposures, the decrease might not be attributable to the effects of the gases - at least not entirely.

Neither combination of 65 ppm NH_3 with 20 or 150 ppm H_2S appreciably altered the effects of H_2S alone. However, when 150 ppm NH_3 was mixed with H_2S, changes were noticeable. Rates for the 20 ppm H_2S - 150 ppm NH_3 combination held constant over both the pre and gas periods but dropped 2/min during post-exposure. This response suggests that the actions of H_2S and NH_3 are probably additive.

The only substantial decrease in respiratory frequency occurred with the 150 ppm NH_3 - 150 ppm H_2S combination. Rates were depressed from a pre-exposure norm of 87/min to a gas-period average of 63/min. Since this change was drastic in comparison to the other treatments, possibly H_2S and NH_3 at high levels interact synergistically to intensify an assault on the respiratory system. Frequencies for the 150 ppm NH_3 - 150 ppm H_2S exposure decreased during day 1 of gassing and a low of 52/min was recorded on day 7. Although degree of depression appears to

correspond with time exposed, the irregular decline, as well as the comparable variation in rates exhibited by the control calves, leaves any proposal of an apparent trend in daily response without foundation.

That the various gas treatments did not significantly affect respiratory rate is affirmed by the analysis of variance (Appendix 5). Only the interaction of days and periods was significant ($P < 0.001$). A discussion of this variation is meaningless because of the erratic and inconsistent fluctuations in daily rates; also, similar daily trends were exhibited by all treatments, including the control.

Hays (58) has suggested that respiration rate per se may not necessarily be a reliable means for quantifying respiratory efficiency. Results in this study would tend to support this view, especially at the lower exposure levels of NH_3 and H_2S. However, Sallvik (109) reported that respiratory rates of cows were considerably higher when the H_2S concentration in the respiration area was about 1 ppm; for some cows, rates increased as much as 25/min above a normal value of approximately 30/min. As mentioned previously, the 20 ppm H_2S treatment caused an apparent increase in frequency, suggesting that one effect of H_2S at these low levels may register as an increase in respiratory rate. Hays (58) reported that frequencies decreased significantly in goats exposed to 100 ppm H_2S, which agrees with the trend observed for the calves at 150 ppm H_2S. Perhaps an inflection point exists where the effect of H_2S on the respiratory centers in the brain changes from stimulation at lower concentrations to depression at higher concentrations.

Variations in response similar to those noted for H_2S were indicated at the low and high levels of NH_3. That is, rates appeared

to be relatively unaffected or even increased at the low concentrations, but were slightly reduced at the high concentrations. This response may be enlightened by recalling that the severity of irritant action does not vary in proportion to gas concentration multiplied by the duration of exposure. A high concentration for even a short time has an intense effect, while reducing the concentration by one-half allows an irritant to be withstood for much more than twice as long with less effect (60). Consequently, the high levels of H_2S and NH_3 probably resulted in a more than proportionate assault on the membranes of the respiratory tract. Therefore, NH_3 in particular may have had little effect at the low level, whereas a reduction in respiratory rate at the high level could have been due to irritation of the upper respiratory tract, causing subsequent constriction of the larynx and bronchi.

Hydrogen sulfide, being less soluble than NH_3, has a more extensive locus of action in the respiratory tract. Hence the lungs may be severely irritated. The substantial decrease in respiratory rates observed for the 150 ppm NH_3 - 150 ppm H_2S treatment may be due to the combined attack of the two gases, resulting in pulmonary edema. This condition seriously interferes with exchange of O_2 and CO_2. Since less edematous areas tend to be hyperaerated, the CO_2 level in the blood may be reduced below normal, inducing an almost proportional decrease in volume of breathing (60).

Resting respiratory frequencies for cattle in the 300 to 1100 lb weight range are 30 to 34/min (119). The average pre-exposure respiratory rate for all calves was 80/min, indicating that most animals were stressed to some degree during this period and hence over the entire trial. Consequently, even though abnormal respiratory

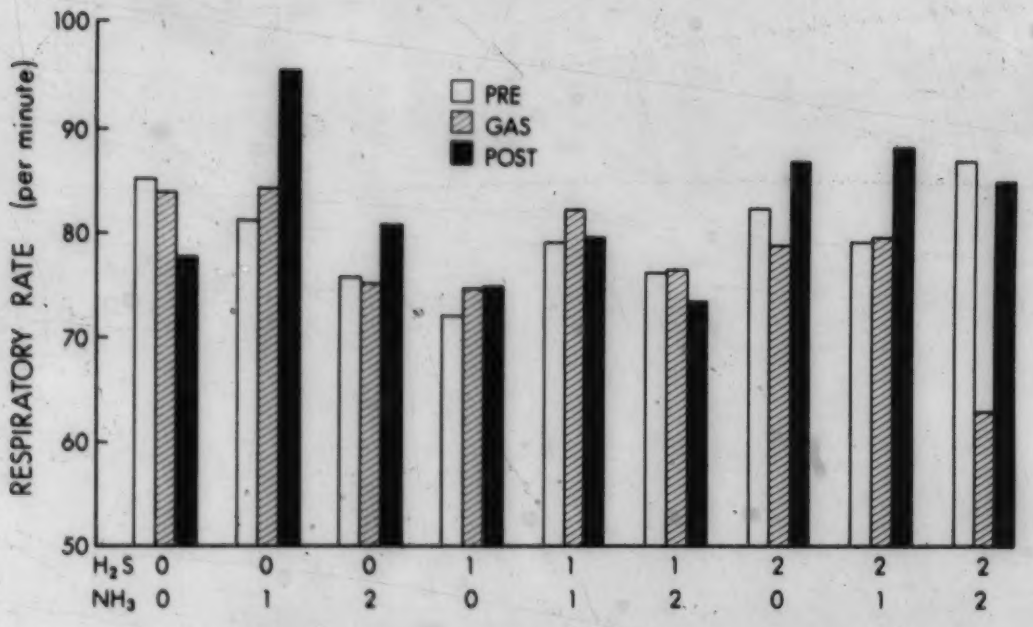

Figure 28. Period means of respiratory rates per calf.

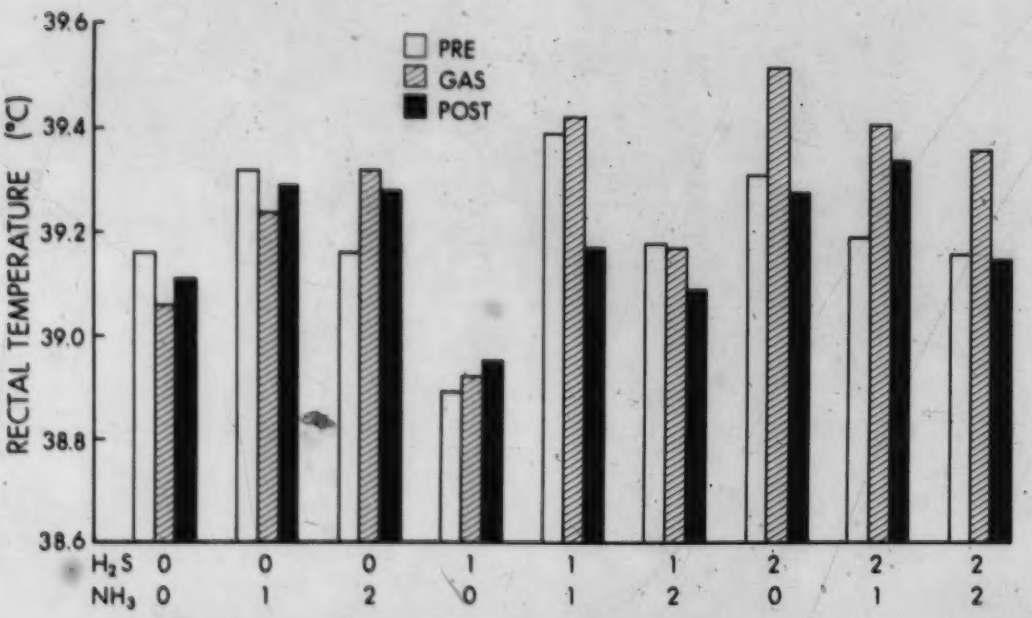

Figure 29. Period means of rectal temperature per calf.

patterns definitely were suggested from visual appearances during the gas-exposure period, many changes may not have been reflected distinctly in the already elevated and unsteady respiratory rates.

4.2 Rectal Temperature.

Rectal temperature data are given in Table 16. Figure 29 illustrates the mean temperatures for each gas treatment and exposure period. During the experiment, the control calves maintained an average body temperature of 39.11°C within a range of 0.10°C, indicating that background NH_3 levels had little or no effect on homeothermy. Similarly, the mean temperatures of calves exposed at 65 ppm NH_3 changed by less than 0.10°C over the three periods. However, the highest daily average (39.55°C) was recorded on day 4 of gas exposure while the lowest temperature (39.00°C) occurred on day 7 of the same period. The 150 ppm NH_3 treatment caused a comparatively large increase in temperature. Pre- and gas-period means were 39.16° and 39.32°C respectively, with a peak value of 39.50°C being recorded on day 4 of gas exposure. Temperature had declined to almost normal by day 7. These responses suggest that NH_3, particularly at 150 ppm, did affect body temperature during the initial four days or so, but the animals were capable of attaining near normal levels within the following three days.

The effects of 20 ppm H_2S alone did not appear to be appreciable. Mean temperatures increased by only 0.02°C during successive periods. The mixture of 65 ppm NH_3 with 20 ppm H_2S caused temperatures to climb no more than did the lone H_2S treatment. However, the highest (39.78°C) and lowest (39.08°C) temperatures occurred during the gas period on days 4 and 7 respectively. The combination of 150 ppm NH_3 and 20 ppm H_2S had essentially no effect on the gas-period mean value. Again though, the highest daily temperature

TABLE 16: MEANS OF RECTAL TEMPERATURES (DEGREES CELSIUS/CALF).

Treatment H_2S	Treatment NH_3	Period Pre	Period Gas	Period Post
0	0	39.16	39.06	39.11
0	1	39.32	39.24	39.29
0	2	39.16	39.32	39.27
1	0	38.89	38.92	38.95
1	1	39.39	39.42	39.17
1	2	39.18	39.17	39.09
2	0	39.31	39.52	39.28
2	1	39.19	39.41	39.34
2	2	39.16	39.36	39.15
		S.E.M. = 0.07		
0		39.22	39.21	39.22
1		39.15	39.17	39.07
2		39.22	39.43	39.26
		S.E.M. = 0.04		
	0	39.12	39.17	39.12
	1	39.30	39.36	39.27
	2	39.17	39.28	39.17
		S.E.M. = 0.04		
		39.20	39.27	39.18
		S.E.M. = 0.02		

Treatment H_2S	Treatment NH_3	Day of Period 7	2	4	7
		Pre	Gas Exposure		
0	0	39.15	39.08	39.10	38.92
0	1	39.18	39.25	39.55	39.00
0	2	39.18	39.42	39.50	39.20
1	0	38.80	39.00	38.95	38.92
1	1	39.28	39.58	39.78	39.08
1	2	39.08	39.32	39.20	39.08
2	0	39.30	39.85	39.75	39.20
2	1	39.05	39.55	39.70	39.32
2	2	39.08	39.55	39.58	39.22
			Post Exposure		
0	0		39.15	38.98	39.40
0	1		39.22	39.52	39.42
0	2		39.25	39.25	39.38
1	0		39.05	38,88	38.95
1	1		39.25	39.18	39.18
1	2		39.15	39.08	39.08
2	0		39.32	39.35	39.25
2	1		39.38	39.25	39.40
2	2		38.90	39.18	39.30
				S.E.M. = 0.13	

was recorded during the gas period. The increase from 39.08°C on day 7 of pre-exposure to 39.32°C on day 2 of gas-exposure suggests that this treatment had some effect on body temperature. Somewhat unexpectedly, the latter H_2S - NH_3 combination apparently had less effect on body temperature than did either gas level alone. This would suggest that the combined effect of 20 ppm H_2S and 150 ppm NH_3 is, at most, one of simple addition.

Either alone or in combination with NH_3, 150 ppm H_2S consistently elevated mean rectal temperatures for the gas period. The treatment with H_2S alone at this level recorded pre- and gas-period values of 39.31° and 39.52°C respectively. The 150 ppm H_2S - 65 ppm NH_3 mixture caused an increase from a pre-exposure norm of 39.19°C to an average 39.41°C over the gas period, while the high level combination had corresponding values of 39.16° and 39.36°C. The equivalent effects of the three treatments, as illustrated by the similar temperature increases between the pre and gas periods, supplies additional evidence that synergism does not occur with H_2S and NH_3. Furthermore, this suggests that H_2S was the predominant factor affecting rectal temperature.

Following the same general trend noted previously for the 65 and 150 ppm NH_3 treatments and for the 65 ppm NH_3 - 20 ppm H_2S combination, highest temperatures during the 150 ppm H_2S treatments were recorded near or on day 4 of gassing, but approached normal by the last day of gas exposure. Thus, there appears to be a thermal control mechanism that has the ability to compensate for the effects of the gases and subsequently return body temperature to normal.

Where temperatures were elevated by gas exposure, some cases

exhibited post-exposure means below those of the pre-exposure normal, while others showed the opposite tendency. There appeared to be no consistency in post-exposure response as associated with treatment.

With reference to the analysis of variance (Appendix 5), a significant difference ($P < 0.05$) between period mean values can be largely attributed to the interaction of periods and H_2S levels, as shown in Figure 30. This significant ($P < 0.05$) interaction, in turn, is primarily due to the effects of 150 ppm H_2S which caused a relatively appreciable rise in rectal temperatures when alone and in combination with both levels of NH_3. Figure 30 also illustrates an interesting trend pertaining to the aforementioned apparent ability of the calves to recover normal body temperatures. As hypothesized, at 150 ppm H_2S the compensatory mechanism established post-exposure temperatures near the pre-exposure level. On the other hand, calves exposed to 20 ppm H_2S exhibited post-exposure temperatures below the pre-exposure norm. Perhaps the slight increase in body temperature during exposure stimulated the recovery mechanism to over-compensate, causing sub-normal temperatures. A subsequent re-adjustment of thermal equilibrium may partly explain the rise in temperatures during the post-exposure period (Figure 31).

Days, and the interaction of days with periods were highly significant ($P < 0.001$) sources of variation. The response curve of daily average temperatures for the exposure periods (Figure 31) illustrates the general trend of temperatures during the gas period to increase up to day 4 and subsequently decline back to or below normal by day 7. From previous discussion, the pronounced changes occurring over the gas period are probably due again to the 150 ppm H_2S treatments.

Cranston et al, cited by Hays (58), have reported that fever in

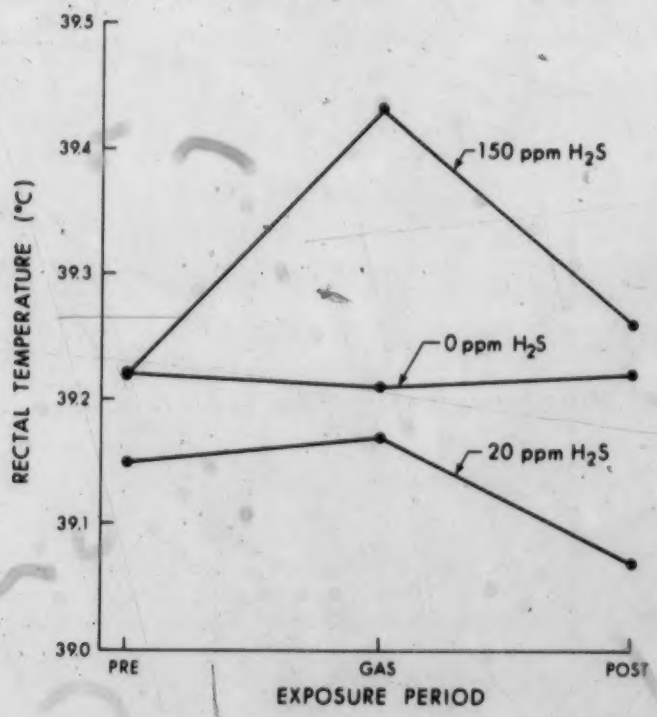

Figure 30. Interaction of exposure period and hydrogen sulfide concentration on rectal temperature.

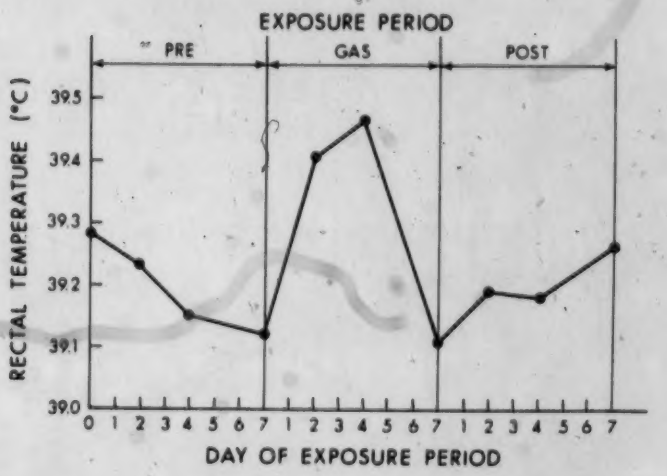

Figure 31. Interaction of days and exposure period on rectal temperature.

mammals is very common with the inflammatory process associated with infection, antigen-antibody reaction or necrosis. As expounded by Hays (58), endogenous and bacterial pyrogens affect the thermoregulatory apparatus of the hypothalamus. Likely, exposure to H_2S and NH_3, particularly at the high levels, produces irritation which then could be responsible for the increased body temperature. Since H_2S is also a systemic poison, its effect on the hypothalamic "set-point" could alter the state of homeothermy. A counter-adjustment in output from this control center may explain the apparent recovery of normal body temperatures.

Although temperatures did not reach the 40.5 to 41.7°C range recorded in most cattle during the initial stages of respiratory disease (57), average daily temperatures for some treatments surpassed 39.7°C - a value which, according to Hanson (57), indicates a fever. This would suggest inflammation of the respiratory tract and a probable lowering of bodily resistance - factors that would be expected to predispose a calf to respiratory infections. Body temperature, then, would appear to be a reliable and effective indication of hazardous exposures to higher levels of H_2S (150 ppm) such as might occur when liquid manure is agitated in a cattle facility.

5. Hematological Data.

5.1 Blood Chemistry.

5.1.1 Sulfhemoglobin.

In the presence of oxygen, hemoglobin reacts with H_2S to form a greenish compound called sulfhemoglobin (39). Whether oxyhemoglobin or other derivatives of hemoglobin react directly with H_2S remains obscure. Apparently, sulfhemoglobin cannot transport oxygen nor be

reduced back to hemoglobin; it remains in the corpuscles until they break down (39).

Laug and Draize (75) have asserted that the blood of rabbits whose skin was exposed to NH_4HS carried from 10 to 20% saturation with sulfhemoglobin. However, tests conducted for all treatments and periods during Replicate 1 to detect sulfhemoglobin in the blood of the calves were negative. This suggests that H_2S may not react with hemoglobin in the blood of cattle to form sulfhemoglobin. At least, analysis for sulfhemoglobin does not appear to be a reliable indication of exposure to H_2S alone or in combination with NH_3.

5.1.2 Nitrogenous Substances.

5.1.2.1 Ammonia.

The relevance of blood NH_3 to veterinary clinical pathology has been discussed by Medway et al (89). This is a highly toxic compound and is usually in very low to non-detectable levels in mammalian blood plasma. Normally, NH_3 is absorbed into the blood from the intestine and carried to the liver where it is converted to urea. In ruminants fed high levels of urea, absorbed NH_3 and the resultant alkalosis has been suggested as the cause of death. Moreover, when the liver is unable to detoxify the NH_3, hepatic coma results.

In this experiment, blood was tested to discover whether atmospheric NH_3 might be absorbed into the circulation, thereby possibly upsetting the acid-base regulating mechanism. Elevated NH_3 levels also have been described for animals in shock (39).

Mean blood NH_3 values for the samples taken during the exposure periods are given in Table 17. As shown in Figure 32, most treatments, including the control, caused similar changes. The general trend was

TABLE 17: SAMPLE MEANS OF BLOOD AMMONIA AND BLOOD UREA NITROGEN.

Treatment H_2S	Treatment NH_3	Ammonia (µgN/100 ml) Pre	Gas Day 2	Gas Day 7	Post Day 7	Urea N (mg/100 ml) Pre	Gas Day 2	Gas Day 7	Post Day 7
0	0	80.8	120.0	144.5	98.2	7.78	11.42	8.75	12.25
0	1	104.5	106.2	136.8	118.0	6.62	8.88	11.12	11.50
0	2	93.8	132.5	157.2	93.2	9.55	12.38	11.62	11.25
1	0	117.5	122.8	101.0	108.0	7.55	10.88	7.75	10.50
1	1	80.8	119.5	151.5	113.0	8.00	9.25	9.50	12.50
1	2	105.8	94.8	139.5	98.2	8.72	12.25	13.12	8.75
2	0	92.8	89.8	172.5	112.2	9.98	13.80	8.75	10.62
2	1	84.5	125.8	141.2	122.5	8.75	9.75	12.12	7.75
2	2	112.5	152.0	183.5	135.2	8.25	13.08	11.45	11.38
		S.E.M. = 24.9				S.E.M. = 2.10			
0		93.0	119.6	146.2	103.2	7.98	10.89	10.50	11.67
1		101.3	112.3	130.7	106.4	8.09	10.79	10.12	10.58
2		96.6	122.5	165.8	123.3	8.99	12.21	10.78	9.92
		S.E.M. = 14.4				S.E.M. = 1.21			
	0	97.0	110.8	139.3	106.2	8.43	12.03	8.42	11.12
	1	89.9	117.2	143.2	117.8	7.79	9.29	10.92	10.58
	2	104.0	126.4	160.1	108.9	8.84	12.57	12.07	10.46
		S.E.M. = 14.4				S.E.M. = 1.21			
		97.0	118.1	147.5	110.0	8.36	11.30	10.47	10.72
		S.E.M. = 8.3				S.E.M. = 0.70			

for blood NH_3 to be lowest at the pre-exposure sampling, to increase successively on days 2 and 7 of gassing, and to drop by day 7 of post-exposure. Thus, although the means of the four samples were highly significantly different (Appendix 6), the source of variation cannot be attributed to any particular treatments.

The results might suggest that blood NH_3 values were sensitive to all exposures, even to the background NH_3. However, the sudden decline for the post-exposure control sample would not appear consistent with the previous increases in blood content of NH_3. Furthermore, the responses were not proportional to gas concentration as may be expected if atmospheric NH_3 did have a direct effect on blood NH_3. Since NH_3 content apparently was affected more by the sampling date and replicate (Appendix 6) than by the gas treatments, changes in blood values are most logically accounted for by the controlled fluctuations of homeostasis or by discrepancies in blood collection and/or analysis techniques. Notably, the results for Replicate 1 demonstrated profound increases during the exposure period while those for Replicate 2 were neither appreciably affected nor did they exhibit comparable trends. A review of procedural methods failed to reveal any errors or reasons for inconsistencies. Nevertheless, the significant variation between replicates and the similar responses for all treatments forego the results as inconclusive and suggest that blood NH_3 may not be a reliable index of exposure to sublethal levels of H_2S and NH_3 gases.

5.1.2.2 Urea Nitrogen.

Of the total non-protein nitrogen (NPN) in whole blood, the chief component responsible for most of the fluctuations seen is urea, which by itself may account for 40 to 50% of the total. Urea is produced by mammals in the liver as the end product of protein catabolism and is eliminated from the body chiefly through the kidneys.

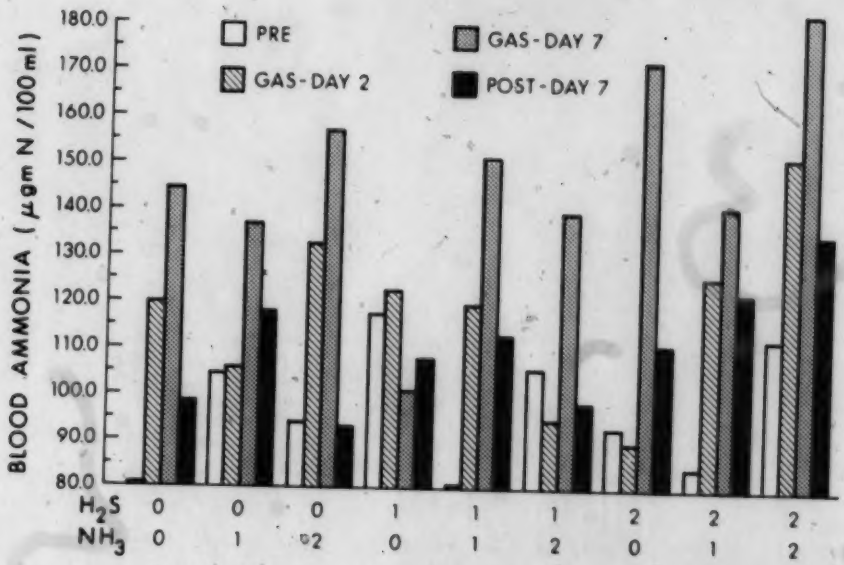

Figure 32. Sample means of blood ammonia.

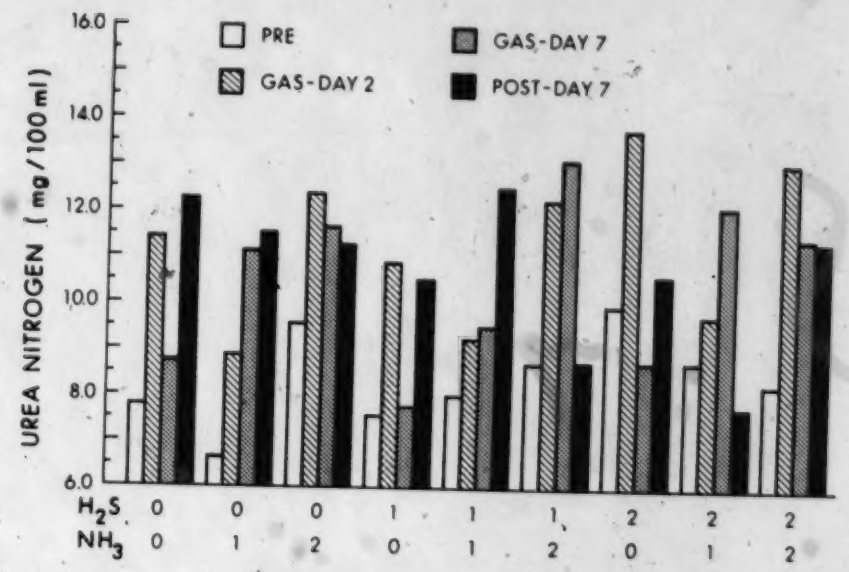

Figure 33. Sample means of blood urea nitrogen.

In ruminants on a low protein diet, blood urea is recycled into the rumen. Elevations in NPN content of blood may be due to cases of renal insufficiency, fever, dehydration and cardiac failure. In all probability, the elevation in NPN can be related to elevation of blood urea nitrogen (BUN)(89). Since the clinical cases listed above have been referred to in reports of animal exposures to manure gases, urea nitrogen appeared to be a potentially useful parameter to investigate. At present, the toxicity associated with uremia generally is believed to be the result of the retention of many waste compounds besides urea, with urea perhaps playing a leading role as a protein denaturant, and in the formation of toxic breakdown products (89).

Blood urea nitrogen values are presented in Table 17 with the fluctuations shown in Figure 33. Although the overall calf averages on the four sampling dates were significantly different (Appendix 6), the gas treatments did not have a significant effect on BUN, indicating that the differences were primarily due to the sampling date and other influences. The significant variation between replicates (Appendix 6) suggests that calf age or body weight may affect BUN concentration. Also, the decreased feed and hence protein intake of calves exposed to the high level gas treatments may have stimulated BUN recycling. However, this cannot account for the increases exhibited by the control calves and others which did not reduce feed consumption. Based on the normal range listed for cattle BUN values, i.e. 6 to 27 mg/100 ml (89), the variations recorded, i.e. 6.6 to 13.8 mg/100 ml, probably were merely reflections of the ever-changing but controlled composition of normal blood.

5.1.2.3 Uric Acid.

Uric acid is the end product of purine and pyrimidine catabolism in animals. The significance of this compound in the blood of mammals is unknown. However, since most domestic animals, including cattle, are capable of converting uric acid to allantoin in the liver, its elevation in the blood has been suggested as an indicator of liver malfunction (89).

The mean uric acid levels for the sampling dates showed similar trends for all treatments (Figure 34). That is, most values were highest at the pre-exposure sampling and lowest on the last day of gassing. Subsequently, the post-exposure samples generally demonstrated increases. Thus, although means on the four successive sampling dates were significantly different (Appendix 7), the responses do not appear to be enlightening nor dependent to any extent upon the effects of the gas treatments. Likewise, the significant interaction between sampling date and H_2S concentration (Figure 35) appears to be due more to the variation of uric acid values on sampling dates other than day 7 of gas exposure. Consequently, these variations are probably normal. The overall mean content of uric acid in the calf serum was 0.56 mg/100 ml while individual samples ranged between 0.25 and 1.00 mg/100 ml (Table 18).

5.1.3 Bilirubin.

Bilirubin, formed in the cells of the reticulo-endothelial system from hemoglobin, is excreted in the bile. As such, elevation of this pigment serves as a test of liver malfunction (39). Productivity is the essence of large animal husbandry, and biochemical methods may be employed to assess the degree of liver function and, hence the economic

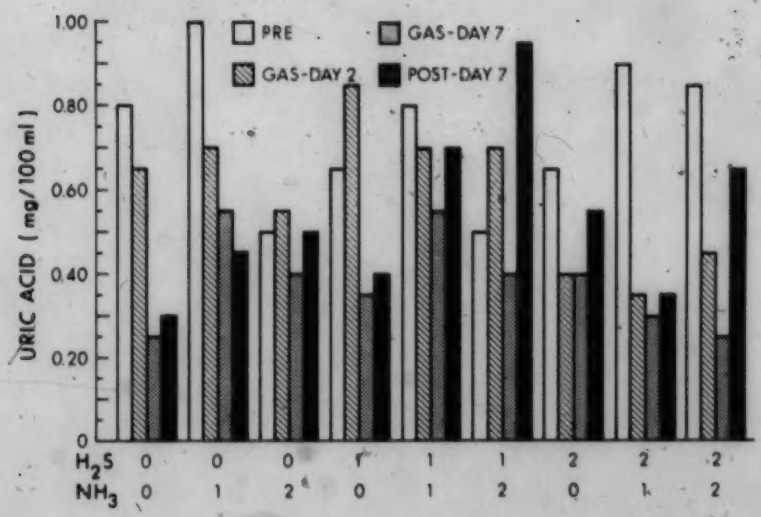

Figure 34. Sample means of serum uric acid.

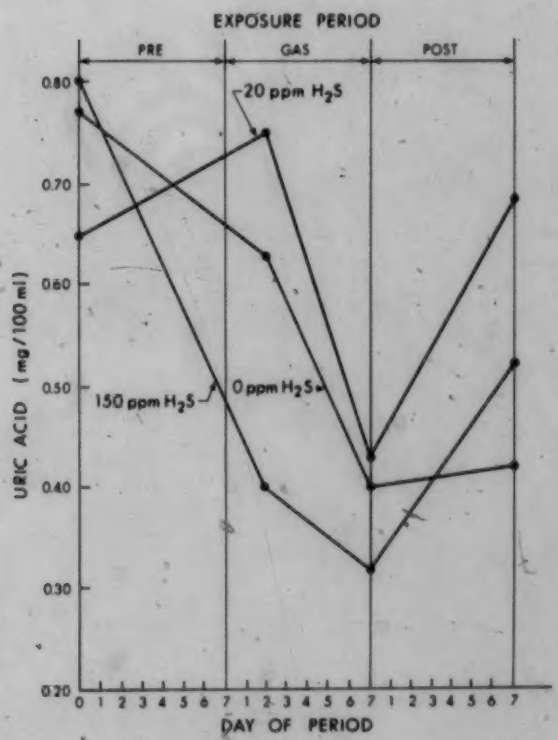

Figure 35. Interaction of sampling date and hydrogen sulfide concentration on serum uric acid.

TABLE 18: SAMPLE MEANS OF SERUM URIC ACID AND TOTAL BILIRUBIN.

Treatment H_2S	Treatment NH_3	Uric Acid (mg/100 ml) Pre	Gas Day 2	Gas Day 7	Post Day 7	Total Bilirubin (mg/100 ml) Pre	Gas Day 2	Gas Day 7	Post Day 7
0	0	0.80	0.65	0.25	0.30	0.238	0.148	0.138	0.032
0	1	1.00	0.70	0.55	0.45	0.372	0.280	0.110	0.282
0	2	0.50	0.55	0.40	0.50	0.230	0.160	0.108	0.005
1	0	0.65	0.85	0.35	0.40	0.268	0.272	0.142	0.020
1	1	0.80	0.70	0.55	0.70	0.268	0.188	0.232	0.048
1	2	0.50	0.70	0.40	0.95	0.225	0.058	0.182	0.022
2	0	0.65	0.40	0.40	0.55	0.282	0.172	0.105	0.005
2	1	0.90	0.35	0.30	0.35	0.270	0.060	0.175	0.068
2	2	0.85	0.45	0.25	0.65	0.230	0.118	0.165	0.078
		S.E.M. = 0.13				S.E.M. = 0.167			
0		0.77	0.63	0.40	0.42	0.280	0.196	0.118	0.107
1		0.65	0.75	0.43	0.68	0.253	0.172	0.186	0.030
2		0.80	0.40	0.32	0.52	0.261	0.117	0.148	0.050
		S.E.M. = 0.08				S.E.M. = 0.096			
	0	0.70	0.63	0.33	0.42	0.262	0.198	0.128	0.019
	1	0.90	0.58	0.47	0.50	0.303	0.176	0.172	0.132
	2	0.62	0.57	0.35	0.70	0.228	0.112	0.152	0.035
		S.E.M. = 0.08				S.E.M. = 0.096			
		0.74	0.59	0.38	0.54	0.265	0.162	0.151	0.062
		S.E.M. = 0.04				S.E.M. = 0.056			

value of an animal. However, in a chronic disease with relatively slow degeneration of liver parenchyma, cellular destruction must be extensive before the health of the animal is impaired; also, the suggestion has been made that the estimation of serum bilirubin does not provide a very sensitive index of liver function (89).

The overall means of bilirubin levels generally declined on successive sampling dates (Table 18), suggesting that liver function of the calves was not impaired. Furthermore, the gas treatments did not have a significant effect on bilirubin (Appendix 6), nor did any sample exceed 0.47 mg/100 ml - the upper limit for normal bovine serum (89). Indications, then, are that bilirubin tests did not assist in evaluating the effects of the various gas treatments in the calves.

5.1.4 Serum Enzymes.

5.1.4.1 Glutamic Oxalacetic Transaminase.

Elevations in the activity of serum glumatic oxalacetic transaminase (SGOT) can be associated with alterations in cell necrosis of many tissues; however, the concentration of this enzyme is higher in muscle cells than in other body cells (89). Pathology involving the skeletal or cardiac muscle and/or the hepatic parenchyma allows for the leakage of large amounts of the enzyme into the blood (73). Serum GOT is the most widely used serum enzyme determination in neuromuscular diseases of domestic animals (74), and significant elevations have been observed in muscular dystrophies of nearly all species (73). Furthermore, experimental work with animals suggests that the degree of rise of serum GOT is related to the extent of myocardial necrosis, while mild to moderate degrees of SGOT elevation have been reported in some human

patients with congestive heart failure and in those with marked tachycardia (39). Elevations of SGOT activities also have been reported in white muscle disease of lambs and calves, and myodegeneration due to ingestion of toxic plants in cattle (74).

Sample means of SGOT values are presented separately for Replicates 1 and 2 (Table 19) since a different laboratory was employed for each replication. Unfortunately, the laboratories used different units to express the amount of enzyme activity per volume of serum. Variations between SGOT values measured during the first replicate were not significant (Appendix 7), and as shown in Figure 36, changes were inconsistent. Although rather sharp increases were registered for the lone 150 ppm NH_3 and the lone 20 ppm H_2S treatments, these trends were not exhibited by the other treatments involving the same gas levels. In fact, the 150 ppm H_2S + 150 ppm NH_3 treatment showed essentially no change in enzyme activity, suggesting that the increases, as related to gas exposure, were meaningless. In accord, the significant sources of variation indicated by the analysis of variance for the results of Replicate 2 (Appendix 7) appear to have no value in interpreting the effects of the gas treatments. Figure 37 illustrates that the only appreciable increase in enzyme level occurred in the control calves. This response, when compared to the other treatments, notably those with 150 ppm H_2S where the most drastic rises, if any, would be expected, appears illogical and perhaps even erroneous. Furthermore, the changes displayed in the two replicates for the same treatments were not compatible (Figures 36 and 37). Consequently, GOT determinations in cattle serum would not appear to be suitable for indexing exposure to manure gases at the concentrations and duration applied in this study.

TABLE 19: SAMPLE MEANS OF SERUM GLUTAMIC OXALACETIC TRANSAMINASE (S.G.O.T.).

Treatment H_2S	Treatment NH_3	S.G.O.T. - Replicate 1 (mIU+/ml) Pre	Gas Day 2	Gas Day 7	Post Day 7	S.G.O.T. - Replicate 2 (TransAc units) Pre	Gas Day 2	Gas Day 7	Post Day 7
0	0	142.5	149.5	154.5	132.0	134.0	170.5	297.0	256.0
0	1	114.5	120.0	151.5	132.5	117.0	110.0	122.0	140.0
0	2	142.5	136.0	254.0	109.0	117.5	155.5	128.0	135.5
1	0	221.0	166.5	234.0	147.0	172.0	144.0	131.0	144.0
1	1	175.5	189.0	178.5	177.5	120.5	145.5	146.5	151.0
1	2	119.5	119.0	120.5	112.5	130.5	141.5	132.5	188.0
2	0	151.5	172.5	190.5	153.5	120.5	116.0	124.0	139.0
2	1	156.0	192.5	198.0	182.5	135.0	169.0	118.0	156.5
2	2	135.0	125.0	126.0	115.5	118.5	115.5	97.5	120.5
		S.E.M. = 36.7				S.E.M. = 12.9			
0		133.2	135.2	186.7	124.5	122.8	145.3	182.3	177.2
1		172.0	158.2	177.7	145.7	141.0	143.7	136.7	161.0
2		147.5	163.3	171.5	150.5	124.7	133.5	113.2	138.7
		S.E.M. = 21.2				S.E.M. = 7.4			
	0	171.7	162.8	193.0	144.2	142.2	143.5	184.0	179.7
	1	148.7	167.2	176.0	164.2	124.2	141.5	128.8	149.2
	2	132.3	126.7	166.8	112.3	122.2	137.5	119.3	148.0
		S.E.M. = 21.2				S.E.M. = 7.4			
		150.9	152.2	178.6	140.2	129.5	140.8	144.1	158.9
		S.E.M. = 12.2				S.E.M. = 4.3			

+ International Units

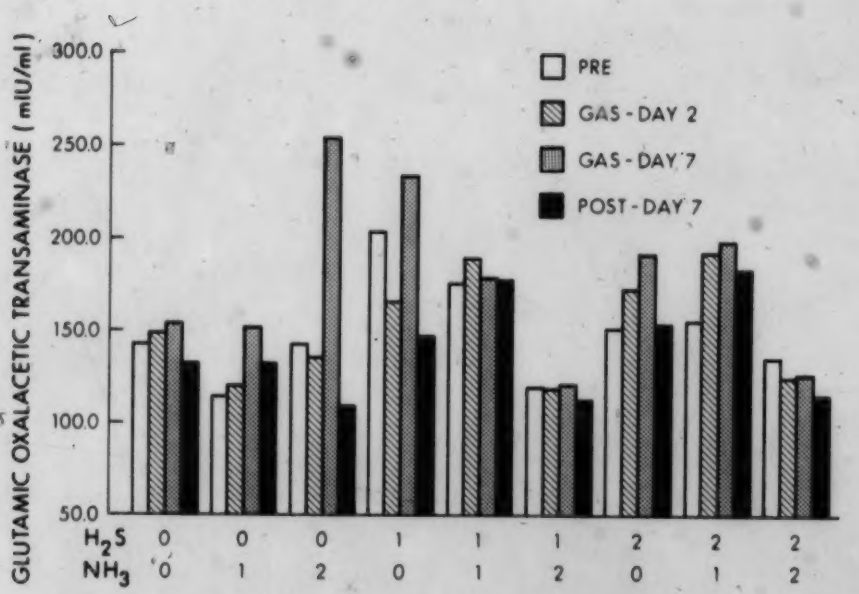

Figure 36. Sample means of serum glutamic oxalacetic transaminase (Replicate 1).

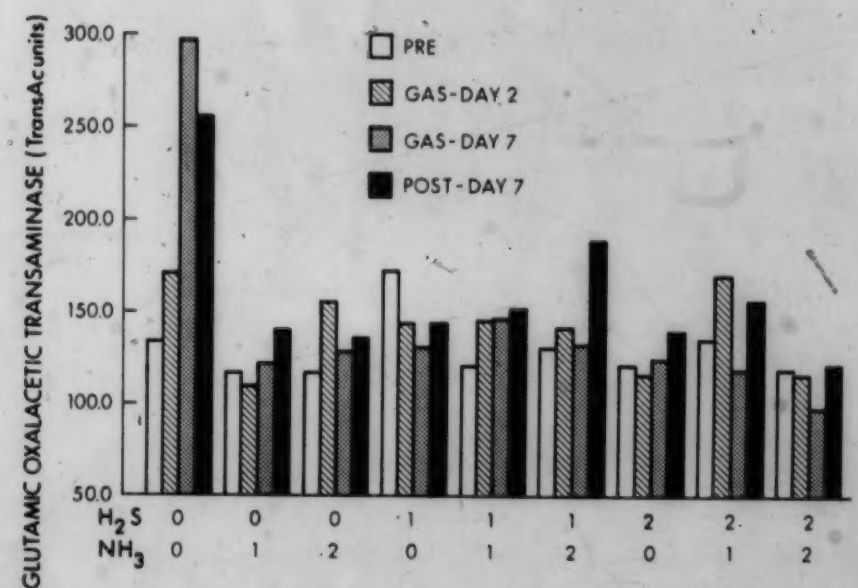

Figure 37. Sample means of serum glutamic oxalacetic transaminase (Replicate 2).

However, the possibility cannot be excluded that cases of different natures may exhibit elevated SGOT levels. Post-mortems on cattle killed by manure gases have revealed extensive hemorrhaging and degenerative processes in muscles and viscera, and encephalitis, while symptoms of chronic conditions have included tachycardia (67).

5.1.4.2 Lactic Dehydrogenase.

Lactic dehydrogenase (LDH) is widely distributed in mammalian tissues, being rich in myocardium, kidney, liver and muscle (39). This enzyme is elevated in almost every process involving necrosis of cells and, therefore, is rather non-specific (89).

The daily means of all samples taken four times during the experiment were significantly different (Appendix 8) and exhibited similar trends for both replicates (Table 20). The enzyme levels were lowest at the pre-exposure sampling date, increased successively on days 2 and 7 of gas exposure, and then dropped slightly by the last day of post exposure. Although the general response appears plausible, comparisons among the individual treatments and between the replications (Figures 38 and 39) cast some doubt on the usefulness of the data. Firstly, the treatments showing the most pronounced LDH elevations were not those that might be expected to cause cell necrosis. For example, while enzyme activity for the calves exposed to the 150 ppm H_2S - 150 ppm NH_3 treatment in Replicate 2 was the lowest on day 7 of gas exposure, the control calves exhibited the greatest increase in LDH. Secondly, response to the same treatment in each replicate was not consistent, except that the more severe treatments, according to gas concentration, did not elevate enzyme levels any more than did the lower gas concentrations. Thirdly, the highest value during the second replicate was

TABLE 20: SAMPLE MEANS OF SERUM LACTIC DEHYDROGENASE (L.D.H.).

Treatment H_2S	Treatment NH_3	L.D.H. - Replicate 1 (mIU/ml) Pre	Gas Day 2	Gas Day 7	Post Day 7	L.D.H. - Replicate 2 (B.B.U.)+ Pre	Gas Day 2	Gas Day 7	Post Day 7
0	0	831.0	775.0	910.0	935.0	1592.5	1577.5	2087.5	1800.0
0	1	760.0	835.0	895.0	855.0	1177.5	1275.0	1430.0	1505.0
0	2	815.0	900.0	1000.0	852.5	1707.5	1652.5	1555.0	1575.0
1	0	798.0	910.0	1015.0	940.0	1812.5	1912.5	1975.0	1777.5
1	1	910.0	975.0	980.0	965.0	1390.0	1512.6	1750.0	1712.5
1	2	730.0	905.0	919.0	860.0	1600.0	1600.0	1632.5	1562.5
2	0	825.0	930.0	912.0	985.0	1262.5	1467.5	1525.0	1480.0
2	1	820.0	945.0	960.0	910.0	1312.5	1537.5	1412.5	1517.5
2	2	822.0	755.0	795.0	760.0	1505.0	1600.0	1445.0	1450.0
		S.E.M. = 39.4				S.E.M. = 92.4			
0		802.0	836.7	935.0	880.8	1492.5	1501.7	1690.8	1626.7
1		812.7	930.0	971.3	921.7	1600.8	1675.0	1785.8	1684.2
2		822.3	876.7	889.0	885.0	1360.0	1535.0	1460.8	1482.5
		S.E.M. = 22.8				S.E.M. = 53.3			
	0	818.0	871.7	945.7	953.3	1555.8	1652.5	1862.5	1685.8
	1	830.0	918.3	945.0	910.0	1293.3	1441.7	1530.8	1578.3
	2	789.0	853.3	904.7	824.2	1604.2	1617.5	1544.2	1529.2
		S.E.M. = 22.8				S.E.M. = 53.3			
		812.3	881.1	931.8	895.8	1484.4	1570.6	1645.8	1597.8
		S.E.M. = 13.1				S.E.M. = 30.8			

+ Berger-Broida units.

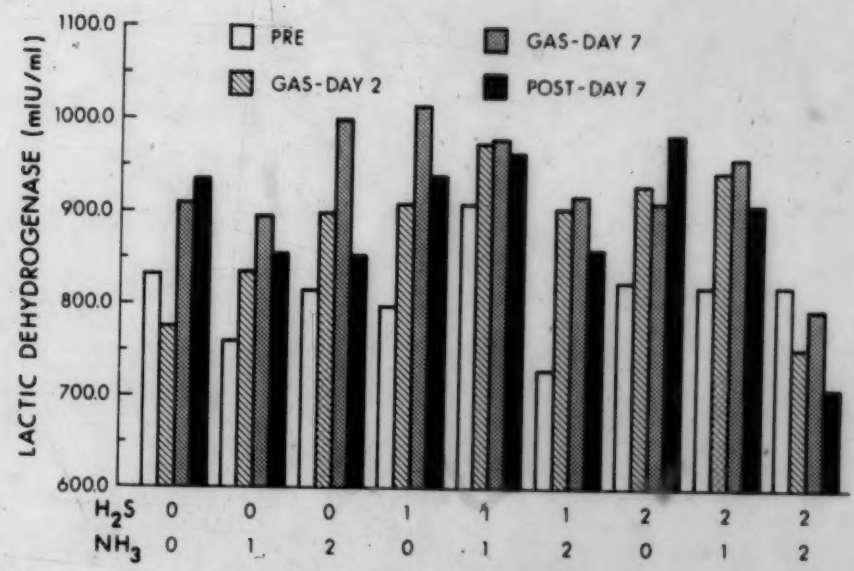

Figure 38. Sample means of serum lactic dehydrogenase (Replicate 1).

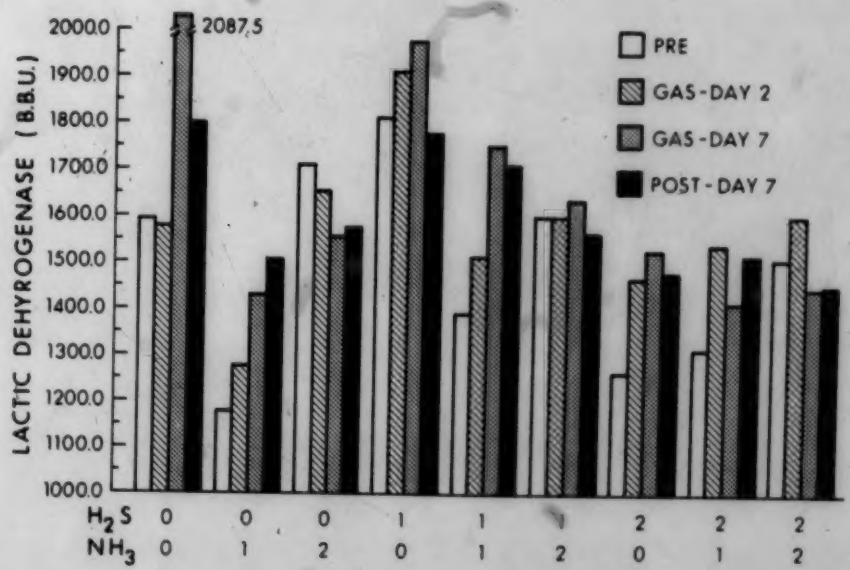

Figure 39. Sample means of serum lactic dehydrogenase (Replicate 2).

recorded for the control calves. Besides being unexpected, the actual value of 2087.5 Berger-Broida units (B.B.U.) was less than 3500 B.B.U., reported as the ceiling level in normal serum of cattle (89). On this basis, the fluctuations in LDH levels cannot be considered abnormal and consequently there would appear to be no grounds to implicate the gas treatments as having any appreciable effect on enzyme activity in the serum. Therefore, the value of LDH in the diagnosis of manure gas exposures similar to those of the experiment appears dubious.

5.1.4.3 Alkaline Phosphatase.

Elevated serum alkaline phosphatase (SAP) levels may occur in any disease process of the spleen, liver, kidney, intestinal mucosa or bones, and also is elevated markedly in biliary obstruction since the enzyme is eliminated in its native form by the liver (89). One difficulty encountered with SAP is the many different units used to express its activity. Consequently, a normal value for cattle was not found. However, analysis of variance procedures (Appendix 9) and an examination of the data did not detect any trends attributable to possible effects of the gas treatments. The grand mean was 105 milli International Units per ml (mIU/ml) and sample values ranged from 75.5 to 146.5 mIU/ml. For reference purposes, the sample means are included in Appendix 9.

5.1.5 Serum Electrolytes.

5.1.5.1 Inorganic Phosphorus.

Concentrations of inorganic phosphorus are presented in Table 21 and shown graphically in Figure 40. The significant difference between average values of all calves for the four sampling dates can be attributed largely to the significant interaction of H_2S levels with

TABLE 21: SAMPLE MEANS OF SERUM INORGANIC PHOSPHORUS.

Treatment H_2S	Treatment NH_3	Inorganic Phosphorus (mg/100 ml) Pre	Gas Day 2	Gas Day 7	Post Day 7
0	0	8.45	8.00	7.25	6.90
0	1	8.15	8.25	8.20	8.60
0	2	7.80	7.90	7.90	8.40
1	0	7.90	8.25	8.30	8.60
1	1	7.20	7.25	7.35	6.90
1	2	8.05	8.20	7.30	8.70
2	0	8.05	7.40	8.15	9.15
2	1	8.60	7.35	7.75	8.60
2	2	8.30	7.05	7.15	8.35
		S.E.M. = 0.32			
0		8.13	8.05	7.78	7.97
1		7.72	7.90	7.65	8.07
2		8.32	7.27	7.68	8.70
		S.E.M. = 0.18			
	0	8.13	7.88	7.90	8.22
	1	7.98	7.62	7.77	8.03
	2	8.05	7.72	7.45	8.48
		S.E.M. = 0.18			
		8.06	7.74	7.71	8.24
		S.E.M. = 0.11			

sampling date (Appendix 8). As shown in Figure 41, the 0 and 20 ppm H_2S treatments exhibited similar responses, with mean phosphorus concentrations declining slightly during the gas period and recovering near pre-exposure levels by the seventh day of post-exposure. However, changes were minor considering that normal concentrations lie in the range 4 to 8 mg per 100 ml for cows, with calves tending to have values about 3 mg per 100 ml higher (89). The 150 ppm H_2S treatments appeared to depress phosphorus from a pre-exposure norm of 8.3 mg/100 ml to 7.3 mg/100 ml by day 2 of gassing. Then, in contrast to the 0 and 20 ppm H_2S treatments, phosphorus content rose to 7.7 mg/100 ml on day 7, and rose again even more sharply during the post-exposure period to 8.7 mg/100 ml on the seventh day. These differential responses to the low and high levels of H_2S suggest that gas exposure may have affected the physiologic control of serum phosphorus. However, whether the observed changes can be considered detrimental to the health of the calves, or meaningful as an indication of disrupted regulation, is questionable since all changes occurred within the normal bounds reported for cattle. The control of plasma inorganic phosphorus is not well established but level is affected by the parathyroid gland, dietary intake, efficiency of absorption, Ca:P ratios and metals; phosphorus deficiency leads to depraved appetite (89). Although only speculative, perhaps the decreased phosphorus levels noted for the 150 ppm H_2S treatments on day 2 of gas exposure caused depressed appetites, while the increased levels influenced the tendency of appetites to recover toward the latter part of the gas-exposure period. On the other hand, the recovery of feed intake may have caused the rise in phosphorus levels.

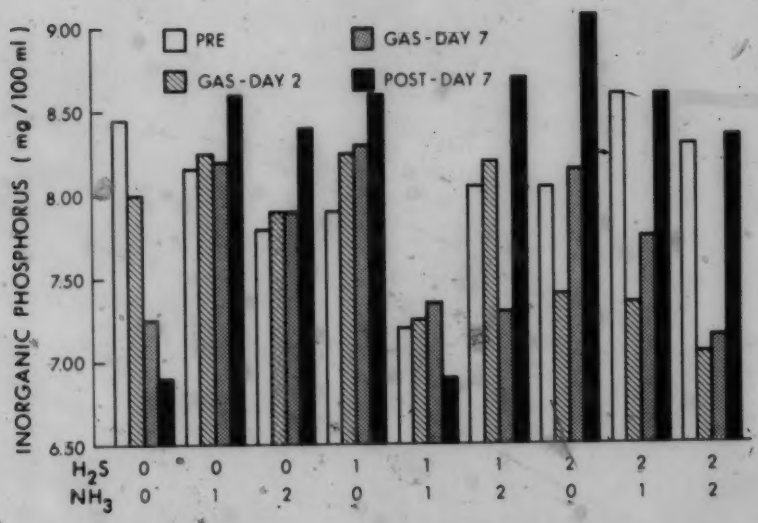

Figure 40. Sample means of serum inorganic phosphorus.

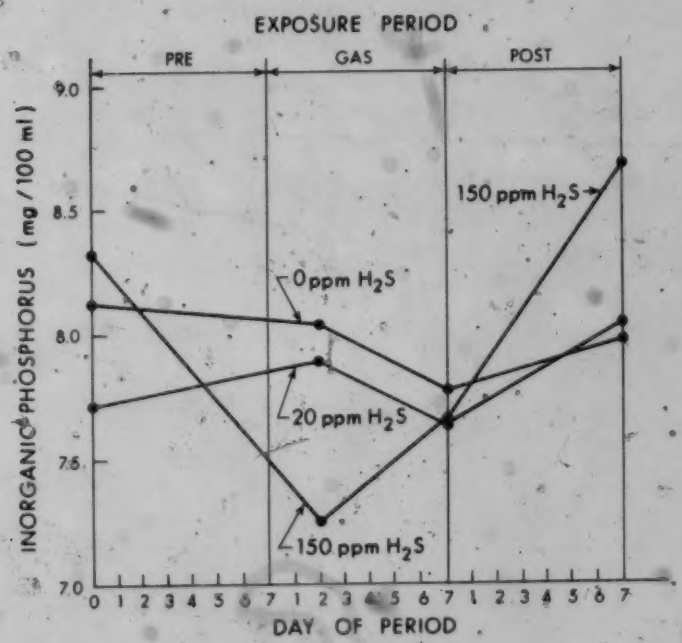

Figure 41. Interaction of hydrogen sulfide concentrations and sampling dates on serum inorganic phosphorus.

5.1.5.2 Calcium.

Analysis of blood calcium levels failed to reveal any trends associated with the gas treatments. However, the sample means and analysis of variance are presented in Appendix 9. Apparently, diurnal fluctuations and day-to-day variations within normal cattle are slight, and values for most animals fall within the narrow range of 9.5 to 11.0 mg/100 ml (89). In close agreement, the overall mean for the replicate was 9.4 mg/100 ml, with individual samples varying between 9.0 and 9.9 mg/100 ml.

5.1.6 Serum Proteins.

5.1.6.1 Total Protein.

The total plasma or serum protein is composed mainly of two large groups, the albumins and the globulins. These and other proteins present in much smaller amounts are affected, more or less, by all disease processes; however, the significance of the fluctuations has yet to be determined in many instances (89). The total protein concentration in serum of cows has been reported as 7.6 g/100 ml (4). Values for the test calves averaged 7.0 g/100 ml and varied between 6.35 and 7.45 g/100 ml. The gas treatments had no apparent effect on protein levels, as demonstrated by the analysis of variance and table of means (Appendix 10).

5.1.6.2 Albumin.

The albumin of blood, as well as all of the globulins, with the exception of some of the gamma globulins, is synthesized in the liver. Therefore, decreased albumin in the blood occurs in fibrosis of the liver while gastroenteritis also may contribute to decreased levels (89). With an average albumin to globulin (A/G) ratio of 0.92, and total protein partitioned into albumin and globulin, the respective

contents of normal cow serum are 3.63 and 3.97 g/100 ml (4). The experimental data did not exhibit any significant changes in serum proteins, but the sample means and analysis of variance are presented in Appendix 10. The average albumin content of all calf samples was 3.34 g/100 ml, resulting in an A/G ratio of 0.91.

5.1.7 Glucose.

The blood glucose level reflects the nutritional, emotional, and endocrine condition of the animal. The concentration does not increase after feeding in ruminants, but does increase during excitement, probably as a metabolic effect of norepinephrine release. Food deprivation tends to diminish the blood glucose concentration slightly (89).

The serum glucose levels, averaged for each sampling date, were significantly different (Appendix 11). The overall mean on day 2 of gassing remained almost unchanged from the pre-exposure norm of 82.2 mg/ 100 ml, but a general increase to 87.7 mg/100 ml occurred by the seventh day of gas exposure,after which the value decreased slightly to 85.4 mg/100 ml on day 7 of post-exposure (Appendix 11). This trend suggests that gluconeogenesis was somewhat stimulated during the gas-exposure period and that the calves might have been mildly excited. However, the absence of any other significant sources of variation implies that the various treatments did not induce markedly different responses, thereby leaving the effects of the individual gas exposures as inconclusive. Notably, though, the combinations of 20 ppm H_2S - 150 ppm NH_3, 150 ppm H_2S - 65 ppm NH_3 and 150 ppm H_2S - 150 ppm NH_3 exhibited the greatest increases and, as such, appear to have contributed the most to the increase in blood glucose on day 7 of

gas exposure.

In spite of this, the maximum and minimum values recorded were 97.5 and 70.0 mg/100 ml respectively. One reference (89) suggested fasting plasma glucose concentrations in the range 40 to 90 mg/100 ml in healthy adult ruminants while another (4) listed 118 mg/100 ml as normal for calf serum. Thus, the fluctuations were not abnormal, even if treatment did have some influence.

5.1.8 Cholesterol.

Although cholesterol is present in all lipid fractions of blood, its importance in disease of domestic animals is not yet established (89). The normal content in the plasma of cattle has been valued as 110 mg/100 ml (4) but a rather wide range from 50 to 230 mg/100 ml also has been reported (89). In this study, neither the analysis of variance nor an examination of the sample means (Appendix 11) revealed any significant changes in cholesterol levels that could be attributable to the gas exposures. The calf serum averaged 75.2 mg/100 ml with extremes of 52.5 and 96.0 mg/100 ml.

5.2 Complete Blood Count.

5.2.1 Erythrocytes.

5.2.1.1 Red Cell Count.

Counting erythrocytes is a procedure abounding with possible sources of errors: errors due to the size of the sample or to the nature of the sample, the operator's error, errors due to equipment, and the so-called unavoidable errors (errors of the field, the counting chamber,and the pipette). For these reasons, the red cell count (RBC) has lost much of its former popularity (39). Summarized data (111) reveal the marked disparity in red cell counts, and indexes derived

therefrom, encountered in published reports on the hematology of cattle. Schalm (111) concluded that until techniques that are free from inherent errors are developed and widely used, to apply normal data of erythrocyte numbers collected in one locality to clinical interpretation elsewhere appears impossible. Even then, regional differences are of such magnitude as to suggest that breed, climate, and nutrition may have a significant influence on the red cell count. However, a range of 5 to 10 x 10^6 erythrocytes per cmm of blood, and an average of 7.0 x 10^6 cells/cmm, are considered normal for nearly all cattle above one year of age (111).

In agreement with the literature, a wide variation in counts was recorded for the test calves (Table 22). In fact, differences between the H_2S - NH_3 interaction means were statistically significant as were differences between mean values on the four sampling dates (Appendix 12). An examination of the effects of individual treatments on RBC, however, suggests that the variations were due to extraneous influences, rather than the gas treatments themselves. For example, the control calves and the calves subjected to the 150 ppm H_2S - 65 ppm NH_3 and 150 ppm H_2S - 150 ppm NH_3 combinations exhibited very similar trends and counts, whereas the greatest changes occurred for the lone H_2S and NH_3 exposures. Thus, response does not appear consistent with treatment and RBC cannot be regarded as a reliable indication of gas exposure. The overall mean count was approximately 4.5 x 10^6 cells/cmm while sample means ranged from 3.0 to 7.3 x 10^6 cells/cmm. These results, when compared to published data, indicate that most of the test calves may have been slightly anemic. This, however, is merely

TABLE 22: SAMPLE MEANS OF RED BLOOD CELL COUNT AND HEMATOCRIT.

Treatment H_2S	Treatment NH_3	Red Cell Count (10^6/cmm) Pre	Gas Day 2	Gas Day 7	Post Day 7	Hematocrit (percent) Pre	Gas Day 2	Gas Day 7	Post Day 7
0	0	3.405	4.555	3.430	3.155	33.5	35.0	35.0	35.5
0	1	6.840	7.335	4.490	3.625	32.5	34.0	37.5	36.0
0	2	6.680	5.375	3.625	3.415	34.0	31.5	33.0	30.5
1	0	7.260	7.235	4.165	3.530	36.0	39.5	36.0	32.5
1	1	3.795	4.275	3.015	3.430	32.5	34.0	34.0	36.0
1	2	4.640	5.245	3.510	3.700	33.5	38.0	37.5	36.0
2	0	5.020	6.760	5.640	3.980	29.0	34.5	42.0	35.5
2	1	3.655	4.685	3.490	3.185	31.0	35.5	38.5	33.5
2	2	3.520	4.165	3.595	3.345	33.0	30.5	32.0	32.0
		S.E.M. = 0.650				S.E.M. = 2.1			
0		5.642	5.755	3.848	3.398	33.3	33.5	35.2	34.0
1		5.232	5.585	3.563	3.553	34.0	37.2	35.8	34.8
2		4.065	5.203	4.242	3.503	31.0	33.5	37.5	33.7
		S.E.M. = 0.375				S.E.M. = 1.2			
	0	5.228	6.183	4.412	3.555	32.8	36.3	37.7	34.5
	1	4.763	5.432	3.665	3.413	32.0	34.5	36.7	35.2
	2	4.947	4.928	3.577	3.487	33.5	33.3	34.2	32.8
		S.E.M. = 0.375				S.E.M. = 1.2			
		4.979	5.514	3.884	3.485	32.8	34.7	36.2	34.2
		S.E.M. = 0.047				S.E.M. = 0.7			

speculation.

5.2.1.2 Hematocrit.

Hematocrit, the volume of erythrocytes expressed as a percentage of the volume of whole blood in a sample, is recognized as the most accurate method available for the detection of anemia (39). The normal range for the packed cell volume (PCV) in the blood of cattle is 24.0 to 46.0% while the mean values lie between 34 and 38% (111).

The hematocrit means (Table 22) were significantly different on the four sampling dates (Appendix 12). However, the lack of statistically significant effects of the gases would suggest that all the calves reacted in a similar manner; in other words, the variations were not attributable to treatment. The average of all samples was 32.8% at pre-exposure and increased to 34.7 and 36.2% on days 2 and 7 of gassing respectively. The mean on day 7 of post-exposure was 34.2%. Although these results show a general elevation for PCV during gas-exposure, an examination of the individual treatment values failed to reveal consistent trends for either H_2S or NH_3. Apparently no factors caused any appreciable change in hematocrit, as all values were within the normal range mentioned above.

5.2.1.3 Hemoglobin.

A mean hemoglobin value for cattle of 11.09 per 100 ml of blood is common, while the normal range apparently is 8.0 to 15.0 g/100 ml (111). In strict agreement, the overall mean of the test samples was 11.1 g/100 ml and the range was 9.50 to 12.25 g/100 ml (Table 23). Similar to the PCV results, the average figures for the sampling dates were significantly different (Appendix 12), but neither the analysis

of variance nor an examination of the data for possible trends in response indicated that the gas treatments might have had a significant effect on hemoglobin concentration. Again, the changes appear to be non-specific and fairly uniform.

5.2.2 Leukocytes.

5.2.2.1 Total White Cell Count.

The mean total white cell count (WBC) of cattle is between 7000 and 9500 per cmm of blood, but normal values may range from 4000 to 12,000 per cmm (111). Apparently, the gas treatments did not significantly alter the total leukocyte numbers, as illustrated by the analysis of variance (Appendix 13). The overall mean WBC was 6507 per cmm (Table 23), indicating that leukocytosis was not prevalent during the exposure periods. Leukocytosis is usually a response to tissue damage or necrosis produced by inflammation, neoplasia, trauma, or intoxication (89). However, the average WBC of the two calves exposed to 150 ppm H_2S alone, almost reached 18,000 per cmm on the second day of gassing (Table 23). The increase, though, was exhibited by only one calf in which the count exceeded 29,000 per cmm. If the count was not erroneous, as might be suspected, indications are that at least the gas exposure would not appear to be responsible for the abnormally high count. The discrepancy in response between the two calves is one reason. Secondly, a count of 11,100/cmm recorded for the calf in question at the pre-exposure sampling suggests that an increase in leukocyte numbers may have been induced prior to gas exposure. Thirdly, the count dropped to about 9200/cmm by day 7 of gassing, whereas a sustained high count would have been expected if the H_2S had stimulated the apparent rise on day 2 of gas exposure. Furthermore, the other two treatments involving 150 ppm H_2S did not cause a similar reaction. The means of all

TABLE 23: SAMPLE MEANS OF HEMOGLOBIN AND WHITE BLOOD CELL COUNT.

Treatment H_2S	Treatment NH_3	Hemoglobin (g/100 ml) Pre	Gas Day 2	Gas Day 7	Post Day 7	White Cell Count (per cmm) Pre	Gas Day 2	Gas Day 7	Post Day 7
0	0	10.75	11.10	11.00	10.95	4350	4142	4439	4802
0	1	10.75	11.70	11.75	11.10	7644	8376	5970	6224
0	2	11.10	10.60	10.50	9.80	7282	7575	7650	4621
1	0	12.00	12.20	11.25	10.40	6781	6410	6477	5234
1	1	10.60	11.15	10.95	11.40	6181	7268	5382	4609
1	2	10.75	11.75	12.00	11.80	4830	5486	5715	6480
2	0	9.50	11.75	12.25	11.30	8176	17,798	8868	5910
2	1	9.85	11.50	11.70	10.75	7604	5426	4790	4440
2	2	10.90	10.85	10.70	10.10	6732	8976	6536	5058
		S.E.M. = 0.48				S.E.M. = 2212			
0		10.87	11.13	11.08	10.62	6425	6698	6020	5216
1		11.12	11.70	11.40	11.20	5931	6388	5858	5441
2		10.08	11.37	11.55	10.72	7504	10,733	6731	5136
		S.E.M. = 0.28				S.E.M. = 1277			
	0	10.75	11.68	11.50	10.88	6436	9450	6595	5316
	1	10.40	11.45	11.47	11.08	7143	7023	5381	5091
	2	10.92	11.07	11.07	10.57	6281	7346	6634	5386
		S.E.M. = 0.28				S.E.M. = 1277			
		10.69	11.40	11.34	10.84	6620	7940	6203	5264
		S.E.M. = 0.16				S.E.M. = 737			

treatment samples, with exception of the high count just discussed, varied between 4142 and 8976/cmm and hence were within the normal range.

5.2.2.2 Differential Count.

Granulocytes.

As discussed by Medway et al (89), the cells which belong to the granulocytic series can be divided into three main groups - neutrophils, eosinophils, and basophils. Of these, neutrophils comprise the largest group and are the most important from a diagnostic point of view. Neutrophils in the blood compose circulating and marginal pools that are roughly equivalent in size. The marrow reserve is five times the total blood granulocyte pool.

When there is a sudden demand for neutrophils, the marginal pool can supply cells to double the count almost instantly. The marginal pool is responsible for the rise in leukocytes after exercise, excitement, fear and feeding. If continued need for neutrophils occurs because of tissue damage, the large reserve of cells in the bone marrow is responsible for meeting this demand. The neutrophil is responsible for phagocytosis of bacteria and small particulate matter, and functions as a part of the body's first line of defence.

Antigen-antibody reactions mobilize eosinophils at the site and increase the level circulating in the blood. Like eosinophils, basophils are formed in the marrow, but little information is available concerning their kinetics. However, basophils inhibit clotting of blood and lymph, and are decreased in the blood of cattle after stress. The rise in leukocyte count is principally due to the initial outpouring of neutrophils from the marginal pool. During an acute response to tissue damage, the

eosinophil count is depressed but rises as a result of chronic inflammation with protein breakdown and antigen-antibody reactions.

In accord with the total WBC, the analysis of variance (Appendix 13) did not reveal any significant sources of variation for the number of neutrophils. The differential count indicated an overall average percentage distribution of neutrophils of about 27% while the individual sample means varied from 14.5 to 48.0% (Table 24). These figures compare closely to the normal values reported for cattle, i.e. an average and range of 28.0% and 15 to 45% respectively (111).

The apparent absence of eosinophils in the circulating blood during the gas- and post-exposure periods indicates that tissue damage as a result of the gas treatments was not sufficient to elevate these cell numbers. The pre-exposure count revealed 1% eosinophils in each of two calves, otherwise the count was zero. While no basophils were noted in any of the samples taken prior to, or on the second day of gassing, 1 to 2% occurred in a few samples collected on the last days of gassing and post-exposure. This response, if meaningful, is contrary to that expected after cattle have been stressed (89). The normal percentage range for eosinophil and basophil numbers apparently are 2 to 20% and 0 to 20% respectively (111).

Lymphocytes.

Lymphatic tissue is most abundant in young animals up to puberty; therefore, the age of an animal must be considered in evaluation of lymphocyte levels. Young cattle have high levels of lymphocytes (89). Lymphocytes are essential to formation of antibody, can transform into macrophages, and may have the ability to form other leukocytes and erythrocytes in the marrow. Adrenocorticotrophic hormone, and 11-oxysteroids

TABLE 24: SAMPLE MEANS OF NEUTROPHILS AND LYMPHOCYTES.

Treatment H_2S	Treatment NH_3	Neutrophils (percent) Pre	Gas Day 2	Gas Day 7	Post Day 7	Lymphocytes (percent) Pre	Gas Day 2	Gas Day 7	Post Day 7
0	0	26.0	28.5	40.0	48.0	70.0	68.5	54.0	46.0
0	1	23.5	20.5	20.5	21.0	71.5	74.0	72.5	74.5
0	2	22.5	31.0	19.5	25.0	74.0	65.0	74.5	69.5
1	0	20.5	16.5	28.0	27.0	76.0	78.5	67.0	67.5
1	1	14.5	26.0	24.5	33.0	80.0	69.0	66.0	62.5
1	2	24.5	25.0	24.5	25.0	74.5	71.5	71.0	64.0
2	0	28.5	30.5	18.5	23.5	67.0	65.0	75.5	75.5
2	1	22.5	32.0	21.0	36.0	74.0	65.0	76.5	58.0
2	2	29.0	31.0	31.0	38.5	68.0	66.5	66.5	57.5
		S.E.M. = 7.3				S.E.M. = 6.6			
0		24.0	26.7	26.7	31.3	71.8	69.2	67.0	63.3
1		19.8	22.5	25.7	28.3	76.8	73.0	68.0	64.7
2		26.7	31.2	23.5	32.7	69.7	65.5	72.8	63.7
		S.E.M. = 4.2				S.E.M. = 3.8			
	0	25.0	25.2	28.8	32.8	71.0	70.7	65.5	63.0
	1	20.2	26.2	22.0	30.0	75.2	69.3	71.7	65.0
	2	25.3	29.0	25.0	29.5	72.2	67.7	70.7	63.7
		S.E.M. = 4.2				S.E.M. = 3.8			
		23.5	26.8	25.3	30.8	72.8	69.2	69.3	63.9
		S.E.M. = 2.4				S.E.M. = 2.2			

arrest lymphocyte formation and produce lysis of these cells so that in severe stress the lymphatic tissue is depleted and lymphocyte numbers fall. However, lymphocytosis is seen in the recovery phase of viral infections and in chronic inflammations (89).

The analysis of variance results (Appendix 13) showed that the gas treatments did not significantly alter the relative number of lymphocytes. The overall distribution was about 69% and sample means varied from 46.0 to 80.0% (Table 24). These levels were probably normal for the test steers since the average and range for adult cattle are 58.0% and 45 to 75% respectively (111).

Monocytes.

The monocyte develops from reticuloendothelial cells throughout the body. It is capable of ingesting protein break-down products and is thought to phagocytize antigenic substances so antibody formation can commence in plasmocytes. Although scanty information is available concerning the stimuli for formation and factors controlling peripheral blood cell levels, monocytosis is usually a response to chronic inflammation (89).

Although the analysis of variance (Appendix 13) indicated significant sources of variation, the lack of correlation between monocyte levels and observed degree of inflammation suggests that the responses cannot be interpreted as being due to the gas exposures. Furthermore, the overall average (4.4%) and range (1.5 to 8.0%) of sample means (Table 25) are equivalent to the normal values quoted for cattle (111).

5.3 Blood Gases and Hydrogen Ion Concentration.

Mean values for pO_2 and pCO_2 appear in Table 26. The overall period means of the samples taken once during each period were statistically

TABLE 25: SAMPLE MEANS OF MONOCYTES AND BLOOD HYDROGEN ION CONCENTRATION (pH).

Treatment H_2S	Treatment NH_3	Monocytes (percent) Pre	Gas Day 2	Gas Day 7	Post Day 7	pH Pre	Gas Day 6	Post Day 6
0	0	4.0	3.0	4.5	5.5	7.430	7.445	7.430
0	1	5.0	5.5	7.0	4.5	7.450	7.380	7.470
0	2	3.5	4.0	6.0	5.5	7.450	7.290	7.465
1	0	3.5	5.0	5.0	5.5	7.455	7.435	7.455
1	1	5.5	5.0	8.0	4.0	7.375	7.420	7.425
1	2	1.5	3.5	5.0	6.5	7.445	7.460	7.450
2	0	3.5	4.5	6.0	1.5	7.425	7.420	7.425
2	1	3.5	3.0	2.5	6.0	7.415	7.460	7.405
2	2	3.0	2.5	2.0	4.0	7.435	7.465	7.475
		S.E.M. = 1.1				S.E.M. = 0.039		
0		4.2	4.2	5.8	5.2	7.443	7.372	7.455
1		3.5	4.5	6.0	5.3	7.425	7.438	7.443
2		3.3	3.3	3.5	3.8	7.425	7.448	7.435
		S.E.M. = 0.6				S.E.M. = 0.022		
	0	3.7	4.2	5.2	4.2	7.437	7.433	7.437
	1	4.7	4.5	5.8	4.8	7.413	7.420	7.433
	2	2.7	3.3	4.3	5.3	7.443	7.405	7.463
		S.E.M. = 0.6				S.E.M. = 0.022		
		3.7	4.0	5.1	4.8	7.431	7.419	7.444
		S.E.M. = 0.4				S.E.M. = 0.013		

TABLE 26: SAMPLE MEANS OF BLOOD GAS PARTIAL PRESSURES (pO_2 and pCO_2).

Treatment H_2S	Treatment NH_3	pO_2 (mm Hg) Pre	pO_2 (mm Hg) Gas Day 6	pO_2 (mm Hg) Post Day 6	pCO_2 (mm Hg) Pre	pCO_2 (mm Hg) Gas Day 6	pCO_2 (mm Hg) Post Day 6
0	0	29.00	24.70	21.75	40.00	33.20	32.25
0	1	32.00	20.50	25.70	40.30	34.00	29.65
0	2	29.00	22.85	28.50	37.25	33.15	31.50
1	0	29.10	21.65	25.25	39.50	35.45	32.40
1	1	30.85	22.00	23.00	44.50	34.85	36.50
1	2	34.10	27.00	25.15	39.50	32.25	34.65
2	0	33.50	23.20	24.50	39.85	35.00	35.00
2	1	28.75	27.50	21.65	40.00	30.45	32.85
2	2	35.50	24.00	25.00	36.50	32.15	31.25
		S.E.M. = 2.69			S.E.M. = 1.49		
0		30.00	22.68	25.32	39.18	33.45	31.13
1		31.35	23.55	24.47	41.17	34.18	34.52
2		32.58	24.90	23.72	38.78	32.53	33.03
		S.E.M. = 1.55			S.E.M. = 0.86		
	0	30.53	23.18	23.83	39.78	34.55	33.22
	1	30.53	23.33	23.45	41.60	33.10	33.00
	2	32.87	24.62	26.22	37.75	32.52	32.47
		S.E.M. = 1.55			S.E.M. = 0.86		
		31.31	23.71	24.50	39.71	33.39	32.89
		S.E.M. = 0.90			S.E.M. = 0.50		

significantly different (Appendix 14) for both pO_2 and pCO_2 measurements. However, since no other sources of variation were significant, this suggests that all treatments, including the control, exhibited similar trends in response. Hence, apparently the calves did not react differently to the different gas levels or combinations and, therefore, a discussion of the variation among treatments is unwarranted.

As illustrated in Figure 42, the highest pO_2 for each treatment occurred at the pre-gas sampling and then dropped on average from 31.3 mm Hg to 23.7 mm Hg by the sixth day of gas exposure (Table 26). Between this time and day 6 of post exposure, some pO_2 values increased while others fell, resulting in a mean partial pressure for all treatments relatively unchanged from that of the gas-period sample. There appeared to be no consistency in post-exposure changes as associated with treatment. Similarly, the highest pCO_2 values were recorded at the first sampling (Figure 43) and then uniformly dropped from a pre-gas average of 39.7 mm Hg to 33.9 mm Hg on day 6 of gas exposure (Table 26). Again, subsequent changes in values were inconsistent and relatively minor, resulting in a post-gas average of 32.9 mm Hg. Published values on partial pressures of O_2 and CO_2 in the blood cattle appear to be lacking. However, reference to data on human blood may provide an appreciation of normal values that should be comparable to cattle. Human venous blood normally has pO_2 values near 38 to 41 mm Hg and pCO_2 values near 43 to 46.5 mm Hg (4). Apparently, then, the mean partial pressures of both O_2 and CO_2 in the calf blood samples were relatively low.

The concomitant decreases in pO_2 and pCO_2 measurements between the pre- and gas-exposure sampling dates may have been partially due to the elevated respiratory rates exhibited by most calves during the

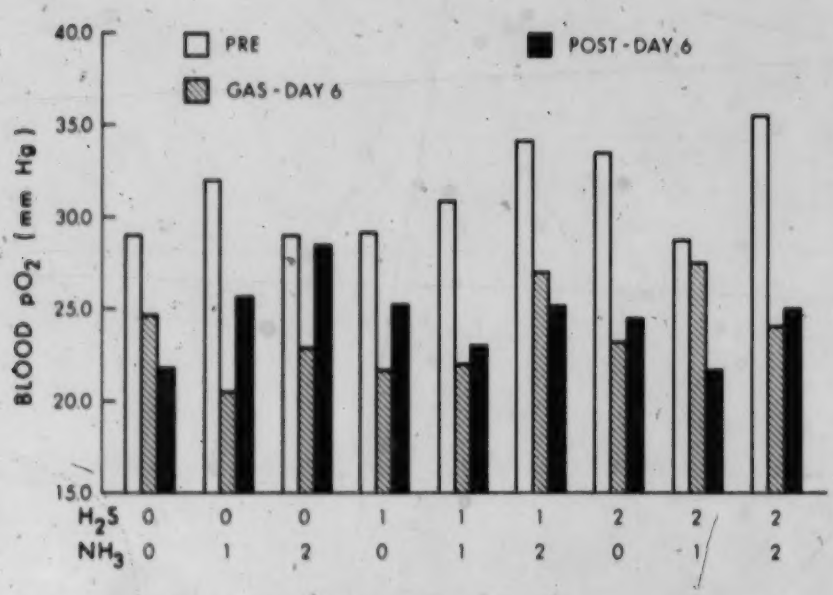

Figure 42. Sample means of blood pO_2.

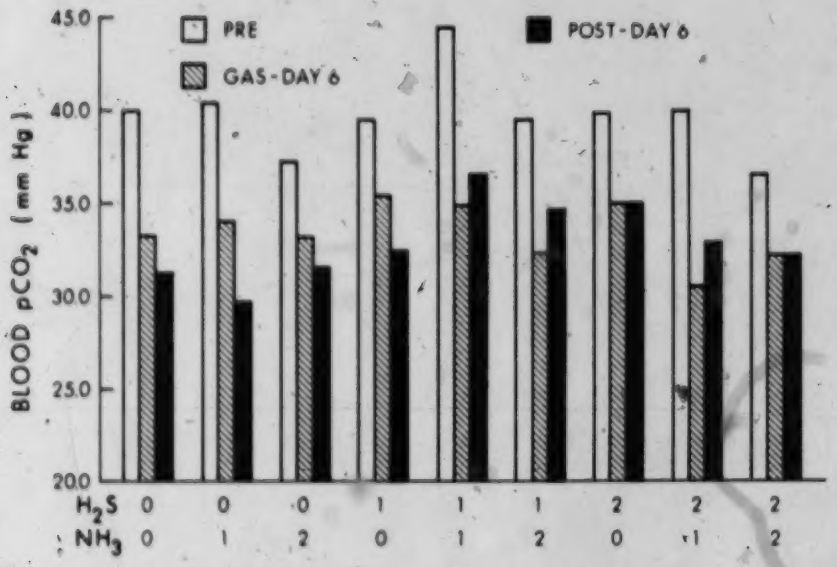

Figure 43. Sample means of blood pCO_2.

experiment. As mentioned previously when discussing respiratory rates, heat stress may have induced panting, or a rapid, shallow form of breathing (polypnea). Irritant effects of the gases, particularly at the high levels, may have accentuated this abnormal respiratory pattern. When resistance to breathing arises from irritation and inflammation, with consequent narrowing of the respiratory airways, expiration is likely to be impeded much more noticeably than inspiration (60). This also tends to induce polypnea by which only a part of the lungs is ventilated. Thus, the fraction of blood flowing through the ventilated area is overaerated and its CO_2 content reduced below normal, while the other fraction of blood passes through the unventilated parts with little or no gain of O_2 or decrease of CO_2. The mixed arterial blood then may have a normal or slightly subnormal content of CO_2; however, the blood is incompletely oxygenated because the overventilated fraction cannot take up more than its hemoglobin allows, while the other fraction is unoxygenated. Pulmonary edema may have similar effects by causing less edematous areas to be hyperventilated.

The comparatively insignificant changes between the gas- and post-exposure periods may reflect a tendency of the calves to acclimatize after two weeks in the chambers. However, the failure of the blood gas partial pressures to regain the pre-exposure values also suggests that other factors may have influenced blood gas content more than the treatments themselves. Whatever the reasons, the similarity in response for all treatments throws doubt on the merit of these blood gas measurements as a sensitive and reliable indication of response to exposure to H_2S and NH_3.

Alterations in plasma pH may be caused by respiratory or

metabolic disturbances. Acidosis, the condition where pH is below normal, may occur in cases of diarrhea, pneumonia, pulmonary edema and pulmonary emphysema. On the other hand, alkalosis may arise from hyperventilation caused by heat stress, hypoxia or hypercapnia, and has been observed in nephritic cows (89). Also, the alkaline properties of NH_3 gas might be expected to upset the bodily pH after prolonged exposure.

However, deviations in pH values of the calf blood samples were statistically insignificant (Appendix 14). The overall mean pH was 7.43 and individual sample means ranged from 7.29 to 7.48 (Table 25). A normal pH value of extracellular fluid is given in most species as 7.40 with a range from 7.30 to 7.50 (89). Therefore, either the gas treatments did not disrupt the acid-base balance of the calves, or any disturbances were compensated so that the pH was corrected quickly. The latter phenomenon may be explained by the tendency of any alteration in plasma pH to activate regulatory mechanisms that subsequently return the pH to as near normal as possible. Hence, on analysis, the pH may be found to be normal with alterations only in the proportions of the salts of the buffer acids (89). This may be the case in respiratory disturbances such as pneumonia or pulmonary edema where limited gaseous exchange may increase the pCO_2 in plasma, causing acidosis. Hypoxia and increased pCO_2 act as respiratory stimulants and the induced hyperventilation limits change in pH.

5.4 Conclusions from Hematological Results.

Although no significant contribution to the diagnosis of exposure to H_2S and/or NH_3 was made in the area of hematology by the foregoing tests, this should not suggest that further efforts cease in this area of research. Since the extensive and routine analysis of blood in the field of veterinary medicine is a relatively new approach to

diagnostic problems, only through continued investigations will the true value of any constituent be known.

Chronic manure gas poisonings in cattle have been reported frequently. However, in a chronic disease, cellular destruction may be slow and must be extensive before the health of the animal is impaired (89). Furthermore, as emphasized by Medway et al (89), there is a predisposition of the blood to promote a stable internal environment. Since many of the parameters that were tested are used as indications of gross clinical diseases, perhaps the tests were not sensitive to the changes that may have occurred during the relatively short exposure period. Also, as demonstrated by the results, different methods designed to measure the same parameter will often give different answers. Similarly, different technicians using the same method may consistently obtain different results (89). On-the-spot analyses by one skilled technician may have provided more accurate and reliable results. The most difficult problem and biggest limitation in interpreting the data was to determine the reason(s) for the changes.

VI. SUMMARY AND CONCLUSIONS

1. From reports cited in the literature review, normal levels of H_2S and NH_3 originating from liquid manure stored within cattle buildings may range from 0 to 20 ppm H_2S and 5 to 30 ppm NH_3. During agitation, measured concentrations at animal level have reached 600 ppm H_2S and 700 ppm NH_3. Furthermore, manure gases do not diffuse and accumulate in the atmosphere at different levels depending on their relative densities. The distribution of gases is determined by different factors dependent to a large extent on the heat production of the animals and on the movement of ventilation air currents.

2. The most prominent clinical symptom of calves exposed to both H_2S and NH_3 was eye irritation, evidenced in the initial stages by inflammation and lacrimation. This appeared to be the principal effect of NH_3 and was usually noticed within the first few days of exposure to either 65 or 150 ppm. Slight corneal opacity was caused by the 150 ppm concentration while additional symptoms included serous nasal discharge that varied from slight at 65 ppm to profuse at 150 ppm, and infrequent dry coughing. Evidence of the irritating effects of NH_3 were no longer readily apparent by the latter days of exposure.

Rather than producing a primary irritant effect as did NH_3, H_2S appeared to have a direct degenerating effect on the viability of exposed membranes of the eye and the nasal mucous membranes. Exposure to 20 ppm H_2S for one week resulted in tissue damage to the cornea that was considered

permanent, even though the lesions would probably heal to satisfactory functional capabilities. At 150 ppm H_2S, severe corneal opacity restricted vision to the point of apparent blindness in extreme cases, and rupture of the cornea appeared possible toward the end of the exposure period. A prominent feature of exposure to 150 ppm H_2S, especially in combination with 150 ppm NH_3, was a tendency of the nasal membranes to hemorrhage. With all H_2S treatments, signs of photophobia were evident, spasmodic coughing was noted, and demeanor varied from lethargy to restless activity. Generally, these overt symptoms lessened by the last one or two days of exposure while affected membranes showed signs of healing by the sixth day of post exposure. No after effects were observed.

3. Exposure to NH_3 alone at low (65 ppm) or high (150 ppm) levels for a period of one week had little or no adverse effect on feed and water consumption. However, feed intake of calves exposed to 150 ppm NH_3 decreased by 16% on the first day of gassing before returning to normal. Hydrogen sulfide alone at 20 and 150 ppm reduced mean feed consumptions during the gas-exposure period by 3.5 and 26% respectively. While exposure to 20 ppm H_2S reduced intake by 14% on day 2, the pre-exposure intake was regained by the fifth day and subsequently surpassed on days 6 and 7. At 150 ppm H_2S, consumption decreased during the first day and dropped a maximum of 40.5% on day 3. Appetites were still 22% below normal on the last

day of gassing, but recovered during day 1 of post-exposure. Although exposure to 20 ppm H_2S did not appreciably affect water intake, at 150 ppm H_2S water intake declined 25.5% on the first day of gas exposure, resulting in a mean period intake that was 75% of normal. When gas infusion ended, the desire to drink recovered immediately.

Results of the NH_3 - H_2S combination treatments suggested that 65 ppm NH_3 did not intensify the adverse effects of H_2S on feed and water consumption. However, the desire to eat and drink appeared to be more severely curtailed when 150 ppm NH_3 was mixed with 20 or 150 ppm H_2S than when either level was infused alone. The 150 ppm NH_3 - 150 ppm H_2S treatment caused the most drastic reduction in consumption; during the gas-exposure period, feed and water intakes averaged 32.5 and 34.5% respectively below the pre-exposure norms. Generally, for all treatments causing anorexia, the effects were apparent during the first day of gas exposure or shortly thereafter. With similar rapidity, in cases where feed consumption was suppressed throughout the gas-exposure period, appetites recovered one or two days after infusion ceased. Similar trends in response were exhibited for water intake.

4. Calves that decreased feed and water consumption during the gas-exposure period, particularly those animals subjected to the treatments involving 150 ppm H_2S, showed an apparent set-back in liveweight gain but displayed compensatory gains over the post-exposure period. To a large extent, liveweight

changes probably reflected differences in feed and water consumption and body fluid content.

5. Respiratory rates appeared to be relatively unaffected or slightly increased at the low levels of H_2S (20 ppm) and NH_3 (65 ppm) while frequencies tended to decrease at the high concentrations (150 ppm) of H_2S and NH_3. However, the only substantial change occurred with the 150 ppm H_2S - 150 ppm NH_3 combination where the average rate during gas exposure was depressed by 24/min from the pre-exposure norm. Respiration rate was not regarded as a reliable means for quantifying the effects of the gases on the respiratory system.

6. During exposure to 65 ppm NH_3, the mean period rectal temperature was relatively unchanged while that of the calves exposed to 150 ppm NH_3 increased 0.16°C above the pre-exposure norm. On the fourth day of gassing, temperatures for the low and high NH_3 treatments rose 0.23 and 0.34°C respectively but declined to near normal by day 7 of gas exposure. Either alone or in combination with NH_3, 20 ppm H_2S had essentially no effect on the gas-period mean values. However, the highest daily temperatures for the 20 ppm H_2S treatments, alone and when mixed with NH_3, were recorded within the first four days of gas exposure and subsequently returned to normal or below by day 7. These results suggested that 20 ppm H_2S affected homeothermy slightly but that its effect was not consistently intensified by combination with 65 or 150 ppm NH_3. Exposure to 150 ppm H_2S alone and in combination with 65 or 150 ppm NH_3 elevated average period temperatures by 0.21,0.22 and 0.20°C

respectively, suggesting that H_2S was the predominant factor affecting rectal temperature. Following the same general trend noted for the aforementioned treatments, temperatures were highest near or on the fourth day of gassing but approached normal by the last day of exposure. Fever was suggested to be an indication of inflammation and respiratory infection - potential effects of prolonged exposure to manure gases.

7. Extensive measurements of blood constituents generally were inconclusive and hence failed to reveal any hematological values that may have been correlated with the toxic effects of H_2S and NH_3. Tests to detect sulfhemoglobin in the blood were negative for all calves. Where changes in values occurred for other parameters, either responses to the treatments were uniform and non-specific, or the variations were within ranges regarded as normal for cattle. Intrinsic sampling and analyzing errors, insensitive tests, slow and limited cellular destruction, the relatively short exposure period, and the ever-changing but controlled fluctuations of homeostasis may have contributed to the difficulties encountered in interpreting the data.

8. The impression gained from these trials was that the apparent detrimental effects of sub-lethal exposure to H_2S and NH_3 were related more to gas concentration than to length of exposure time. This agrees with the literature on the action of irritant gases. Indications were that the effects of H_2S on calf response were similar but more severe and extensive than those of NH_3.

Furthermore, the effects of H_2S and NH_3 in combination usually appeared to be additive; that is, one gas did not intensify the action of the other.

9. The results of this study suggest that desirable threshold limit values for levels of H_2S and NH_3 in cattle buildings would be below the concentrations to which the calves were subjected. While the gas levels that were applied may be encountered in practice under poor ventilation conditions or during pit cleaning operations, even when the ventilation has been assessed as adequate, the effects of very low concentrations of H_2S and NH_3 that may occur above undisturbed liquid manure remain largely unresolved. Thus, the response of cattle exposed continuously to very low levels of manure gases, particularly for periods extending into months, appears to warrant further investigation. However, as astutely pointed out by other researchers (7) and confirmed in this experiment, one of the greatest difficulties experienced in determining the toxicity of low concentrations of most atmospheric pollutants is measurement of the effects. No one criterion, nor even a selected few, is likely to serve as an adequate and specific measure. Because the procedures involved in studying the effects for long periods are unquestionably expensive and tedious, complete resolution of the manure gas problem, by necessity, may have to await multi-discipline research at an established experimental institution.

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APPENDIX 1: GAS FLOWMETER CALCULATIONS.

Since the flowmeters were graduated to provide a direct reading in scfm of air, desired gas flows under actual metering conditions had to be converted to the corresponding air-flow equivalents. The following equation was used to make the required conversion (26):

$$(\text{scfm air equivalent}) = (\text{scfm metered gas flow}) \times \sqrt{\frac{\text{s.g.}}{1.0} \times \frac{To}{530} \times \frac{14.7}{Po}} \quad \ldots\ldots [1]$$

where s.g. = specific gravity of metered gas (air = 1.0)

To = operating temperature absolute (460 + °F)

Po = operating pressure absolute (13.54* + psig)

* Barometric pressure at Edmonton = 700 mm Hg absolute = 13.54 psia.

Specifications

Chamber ventilation rate = 145 scfm

Gas operating conditions:

To = (460 + 70) = 530°F

Po = (13.54 + 10) = 23.54 psia

s.g. of NH_3 = 0.59

s.g. of H_2S = 1.19

Substituting for To and Po in equation [1]:

$$(\text{scfm air equivalent}) = (\text{scfm metered gas flow}) \times 0.79 \sqrt{\text{s.g.}} \quad \ldots\ldots\ldots [2]$$

Inserting the s.g.'s of NH_3 and H_2S in equation [2], the conversion equations become:

$$(\text{scfm air equivalent}) = (\text{scfm metered } NH_3 \text{ flow}) \times 0.61 \quad \ldots\ldots\ldots\ldots [3]$$

$$(\text{scfm air equivalent}) = (\text{scfm metered } H_2S \text{ flow}) \times 0.86 \quad \ldots\ldots\ldots\ldots [4]$$

The gas flows required for the ventilation rate of 145 cfm were calculated as follows:

$$(\text{desired gas concentration}_{\text{ppm} \times 10^{-6}}) \times 145 \text{ cfm} = (\text{scfm metered gas flow}) \quad \ldots\ldots\ldots [5]$$

Conversions

cfm x 60 = cfh

cfh x 472 = cc/min

The final results of the flowmeter calculations are summarized below.

Gas Concentration	Metered Gas Flow	Air Flow Equivalent	Flow meter Air Capacity*
20 ppm H_2S	82 cc/min	71 cc/min	140 cc/min
50 ppm NH_3	0.44 scfh	0.26 scfh	1.2 scfh
150 ppm NH_3	1.31 scfh	0.8 scfh	2.0 scfh
150 ppm H_2S	1.31 scfh	1.1 scfh	2.0 scfh

* Air flows at 14.7 psia and 70°F.

APPENDIX 2: RESULTS OF BLOOD TESTS ON FEEDLOT CALVES.

1. 'Before and after' values to compare changes between sampling times. (All samples taken from same calf.)

Period	Pre	Pre	Gas	Gas	Gas	Post	Post
Day	2	7	2	6	7	6	7
Time before	0830	0830	0900	0800	0830	0800	0830
Time after	0940	1015	1030	0925	0940	0940	0940
B.U.N.	11.2		10.0		10.0		15.8
mg/100 ml	11.5		11.0		12.0		16.0
BILI.	0.00		0.20		0.00		0.00
mg/100 ml	0.12		0.00		0.00		0.13
L.D.H.	1350		1275		1325		1350
B.B.U.	1225		1300		*		1325
S.G.O.T.	126		101		138		149
Trans Ac Units	120		101		151		145
NH_3	46		71		75		100
μg^3N/100 ml	43		69		67		89
pO_2		31.0		24.2		24.0	
mm Hg		31.2		24.2		32.0	
pCO_2		34.0		36.0		33.5	
mm Hg		30.0		38.5		29.8	
pH		7.46		7.42		7.52	
		7.48		7.37		7.52	

* No sample.

2. Samples taken to check consistency of analyses and to compare plastic versus glass syringes for preserving blood gases.

Calf Number	Time	NH_3 µgN/100 ml	B.U.N. mg/100 ml	Syringe	pO_2 mm Hg	pCO_2 mm Hg	pH
112	0850	**	8.0	glass	28.0	35.5	7.44
				glass	28.0	34.5	7.45
				plastic	25.5	36.5	7.44
	1000	86	9.0	glass	27.2	37.8	7.35
				glass	28.2	37.8	7.45
				plastic	29.5	34.0	7.45
114	0900	96	11.1	glass	26.5	32.5	7.46
				plastic	22.2	35.8	7.43
	1015	88	12.3	glass	20.2	34.5	7.40
				plastic	21.0	33.8	7.43

** Sample not valid.

APPENDIX 3: REPORT OF CLINICAL AND GROSS PATHOLOGIC EXAMINATIONS OF CALVES.

Submitted by Dr. B.E. Beck, Animal Disease Section, Veterinary Services Branch, Alberta Department of Agriculture, Edmonton, Alberta.

The four examinations, as reported, correspond to the following numbers:

0. Pre-exposure.
2. Two days after gas turned on.
6. Six days after gas turned on.
13. Six days after gas shut off.

Treatment H_2S	Treatment NH_3	Calf Number		Remarks
0	0	119	0.	Some lacrimation and crusting around left eye.
			2.	Some lacrimation, wet muzzle, but eyes, nose and membranes are normal.
			6.	Slight lacrimation, just detectable dullness of the eye.
			13.	Normal.
		104	0.	Some lacrimal exudate, otherwise normal.
			2.	Normal, no change.
			6.	Normal, no change.
			13.	Normal.
0	1	120	0.	Normal.
			2.	Shows a slightly wet nose, some very mild irritation. Serous discharge.
			6.	Appears normal, there is very slight evidence of lacrimation.
			13.	Normal.
		105	0.	Normal.
			2.	Slight serous nasal discharge and reddening of the mucous membrane.
			6.	Slight lacrimation.
			13.	Normal.

APPENDIX 3: Continued.

Treatment H_2S	Treatment NH_3	Calf Number		Remarks
0	2	113	0.	Normal.
			2.	No lacrimation, very slight opacity, profuse serous nasal discharge with rubifacicience.
			6.	Opacity is present as faint cloudiness and very dull sheen, slight mucous membrane infiltration.
			13.	Eyes bright and normal
		116	0.	Minute papules in lateral dorsum of left nostril.
			2.	Profuse nasal serous discharge.
			6.	Slight opacity, faint cloudiness and a dull sheen.
			13.	Bright and normal.
1	0	107	0.	Normal.
			2.	No change except slight dullness of the eyes and slight cyanosis of the mucous membrane.
			6.	Just detectable opacity at the centre of the eyes with lacrimation.
			13.	Normal.
		102	0.	Normal.
			2.	Definite cloudiness of the cornea, mild lacrimation, mild inflammation of mucous membranes.
			6.	Slight dullness of the cornea, eyes have been lacrimating, there are no definite lesions present.
			13.	Normal, no lesions present.

APPENDIX 3: Continued.

Treatment H_2S	Treatment NH_3	Calf Number		Remarks
1	1	106	0.	Normal.
			2.	Eyes have a dull appearance, there is profuse lacrimation, there is congestion of the mucous membranes, animal is coughing.
			6.	Slight lacrimation.
			13.	Very mild nasal irritation, otherwise normal.
		103	0.	Large papule on the left lateral nares.
			2.	Profuse lacrimation, conjunctiva cyanosed, mucous membranes congested, profuse nasal discharge, coughing.
			6.	Focal opacity 3 mm in diameter at the centre of the cornea, profuse lacrimation.
			13.	Normal.
1	2	115	0.	Normal.
			2.	Mild opacity noted at centre of cornea, mucous membranes are inflamed, there is profuse lacrimation.
			6.	Profuse lacrimation, slight irritation of mucous membranes, lineal central corneal opacity of the right eye.
			13.	Normal.
		118	0.	Opacity in right lens, not the cornea.
			2.	There is profuse lacrimation, inflammation of mucous membranes and slight corneal clouding.
			6.	Slight cloudiness in the right eye, there is lacrimation, squinting and fast breathing. The left eye appears unaffected.
			13.	Normal.

APPENDIX 3: Continued.

Treatment H_2S	Treatment NH_3	Calf Number		Remarks
2	0	109	0.	Slight scurfing around eyelids, normal.
			2.	Corneal opacity, dullness of membranes, lacrimation, strabismus, mucous membranes normal.
			6.	Severe corneal opacity, refuses to open one eye, vision restricted to bright light, bumps into things, mucous membranes look slightly cyanotic but not inflamed.
			13.	Annular vascularization at corneal-scleral junction with hypopyon and mild keratoconus with pannus formation. Where lids don't cover cornea is a more intense whitish mat, looks like a precipitate.
2	0	108	0.	Several macules in right nostril, slightly white and raised.
			2.	Considerable opacity of cornea but not quite as much as animal 109. Mucous membranes are more inflamed in the nose, there are flecks of blood from the nostrils.
			6.	Same corneal opacity as 109, horizontal line of opacity through the centre, mucous membranes are more inflamed. Both these calves appear dopey and lethargic. Opacity is more severe in the center involving that part of the cornea still exposed when the animal squints.
			13.	Right eye shows severe changes as 109, but is limited to more dorsal half. The left eye has mild cloudy infiltrate.
2	1	121	0.	Normal.
			2.	Slight opacity, profuse lacrimation and tenderness, mildly inflamed mucous membranes, little epistaxis, cyanotic mucous membranes.
			6.	Severe opacity, tenderness and pain, right eye more severely affected.
			13.	Apparent lack of vision, ran past door without seeing it, lacrimation and tenderness.

APPENDIX 3: Continued.

Treatment H_2S	Treatment NH_3	Calf Number		Remarks
		110	0.	Very nervous animal, salivating, but no lesions present.
			2.	Marked opacity, lacrimation, nasal discharge, mucous membrane is not inflamed, a small quantity of blood or epistaxis present.
			6.	Severe opacity, but more severe in the centre of the right eye, almost producing keratoconus, left eye is less severe.
			13.	White spots over dull cornea on both eyes. Permanent damage is evident.
2	2	122	0.	Normal.
			2.	Very severe opacity, worse near the medial canthus, the nasal membranes are bright red with hemorrhage or epistaxis.
			6.	Total corneal opacity in both eyes, no area is spared, severe epistaxis, sero-purulent exudate.
			13.	Severe vascularization, opacity, keratoconus in both eyes, membranes give appearance of lack of irritation now as if they are healing.
		123	0.	Papules on lateral dorsal septum, crusts on muzzle from feed.
			2.	Profuse lacrimation from right eye, profuse nasal serous discharge, mucous membranes not too red but there is pronounced epistaxis.
			6.	Just detectable corneal opacity at the centre of the cornea, strabismus, with considerable lacrimation and mucous membrane irritation. Cornea is mostly clear.
			13.	Very slight dullness, otherwise normal left eye; the right eye shows a white spot, approximately 2 x 4 mm, there is considerable lacrimation with tenderness.

APPENDIX 4: ANALYSIS OF VARIANCE: FEED AND WATER CONSUMPTION.

	Feed			Water		
Source of Variation	d.f.[x]	M.S.[xx]	F	d.f.	M.S.	F
H (H_2S levels)	2	73.92	0.92	2	3.60	0.38
N (NH_3 levels)	2	84.20	1.04	2	20.31	2.17
HN	4	11.17	0.14	4	7.20	0.77
R (Replicates)	1	553.89	6.87*	1	100.11	10.71*
Error (1) (HR + NR + HNR)	8	80.64		8	9.35	
C (Calves)/HNR)	18	61.38		18	4.32	
P (Exposure periods)	2	204.91	10.09**	2	11.76	7.86**
PH	4	123.51	6.08**	4	6.66	4.45*
PN	4	6.98	0.34	4	0.68	0.46
PHN	8	14.46	0.71	8	0.69	0.46
Error (2) (PR + PHR + PNR + PHNR)	18	20.30		18	1.50	
PC/HNR	36	20.68		36	0.88	
D (Days)	6	8.87	2.88*	6	1.94	0.09
DH	12	3.20	1.04	12	0.24	0.54
DN	12	2.62	0.85	12	0.69	1.54
DHN	24	5.14	1.67	24	0.27	0.61
Error (3) (DR + DHR + DNR + DHNR)	54	3.08		54	0.45	
DC/HNR	108	2.72		108	0.21	
DP	12	4.51	1.21	12	0.67	2.07*
DPH	24	5.49	1.48	24	0.15	0.47
DPN	24	2.55	0.69	24	0.40	1.25
DPHN	48	4.39	1.18	48	0.36	1.11
Error (4) (DPR + DPHR + DPNR + DPHNR)	108	3.71		108	0.32	
DPC/HNR	212[+]	3.42		211[++]	0.28	

* $P < 0.05$
** $P < 0.01$
\+ 4 d.f. subtracted for 4 missing values estimated using analysis of covariance procedure (117).
++ 5 d.f. subtracted for 5 missing values estimated using analysis of covariance procedure (117).
x Degrees of freedom.
xx Mean squares.

APPENDIX 5: ANALYSIS OF VARIANCE: RESPIRATORY RATE AND RECTAL TEMPERATURE.

	Respiratory Rate			Rectal Temperature		
Source of Variation	d.f.	M.S.	F	d.f.	M.S.	F
H (H_2S levels)	2	2212.7	0.51	2	1.05	2.38
N (NH_3 levels)	2	2456.7	0.56	2	1.10	2.48
HN	4	488.3	0.11	4	0.80	1.81
R (Replicates)	1	254.9	0.06	1	3.59	8.13*
Error (1) (HR + NR + HNR)	8	4348.7		8	0.44	
C (Calves)/HNR	18	4028.0		18	0.54	
P (Exposure periods)	2	1519.1	0.79	2	0.31	4.76*
PH	4	1341.5	0.70	4	0.21	3.19*
PH	4	916.0	0.47	4	0.02	0.32
PHN	8	678.4	0.35	8	0.11	1.68
Error (2) (PR + PHR + PNR + PHNR)	18	1930.1		18	0.07	
PC/HNR	36	677.5		36	0.11	
D (Days)	6	343.2	1.02	3	0.38	8.20***
DH	12	102.1	0.30	6	0.05	1.04
DN	12	95.8	0.28	6	0.09	1.97
DHN	24	116.3	0.34	12	0.05	1.08
Error (3) (DR + DHR + DNR + DHNR)	54	337.2		27	0.05	
DC/HNR	108	130.4		54	0.04	
DP	12	1472.0	3.21***	6	0.59	8.41***
DPH	24	100.2	0.22	12	0.08	1.14
DPN	24	131.0	0.28	12	0.03	0.44
DPHN	48	186.6	0.41	24	0.04	0.61
Error (4) (DPR + DPHR + DPNR + DPHNR)	108	459.0		54	0.07	
DPC/HNR	212+	144.3		108	0.05	

* $P < 0.05$

*** $P < 0.001$

\+ 4 d.f. subtracted for 4 missing values estimated using analysis of covariance procedure (117).

APPENDIX 6: ANALYSIS OF VARIANCE: BLOOD AMMONIA, UREA NITROGEN AND TOTAL BILIRUBIN.

Source of Variation	Ammonia			Urea Nitrogen			Total Bilirubin		
	d.f.	M.S.	F	d.f.	M.S.	F	d.f.	M.S.	F
H (H_2S levels)	2	2780.2	1.04	2	4.06	0.42	2	0.0117	0.33
N (NH_3 levels)	2	1661.5	0.62	2	23.03	2.40	2	0.0520	1.47
HN	4	1520.6	0.57	4	3.89	0.41	4	0.0279	0.79
R (Replicates)	1	210,380.0	78.64***	1	100.50	10.49*	1	1.2063	34.15***
Error (1) (RH + RN + RHN)	8	2675.1		8	9.58		8	0.0353	
C (Calves)/HNR	18	976.1		18	26.94		18	0.0092	
S (Sampling Days)	3	16,354.0	6.60**	3	59.39	6.73**	3	0.2474	4.46*
SH	6	958.1	0.39	6	5.92	0.67	6	0.0144	0.26
SN	6	526.6	0.21	6	20.23	2.29	6	0.0133	0.24
SHN	12	1438.4	0.58	12	7.43	0.84	12	0.0133	0.24
Error (2) (SR + SRH + SRN + SRHN)	27	2476.9		27	8.82		27	0.0555	
SC/HNR	52+	731.0		53++	7.91		53++	0.0070	

* P < 0.05
** P < 0.01
*** P < 0.001
\+ 2 d.f. subtracted for 2 missing values estimated using Yates' method (117).
++ 1 d.f. subtracted for 1 missing value estimated using Yates' method (117)

APPENDIX 7: ANALYSIS OF VARIANCE: SERUM URIC ACID AND GLUTAMIC OXALACETIC TRANSAMINASE

Source of Variation	Uric Acid			S.G.O.T. (Replicate 1)			S.G.O.T. (Replicate 2)		
	d.f.	M.S.	F	d.f.	M.S.	F	d.f.	M.S.	F
H (H_2S levels)	2	0.0893	1.50	2	2186.9	0.88	2	5283.2	3.65
N (NH_3 levels)	2	0.0510	0.86	2	7988.1	3.20	2	6602.2	4.56*
HN	4	0.0401	0.68	4	6773.1	2.71	4	7831.8	5.42*
Error (1) (C(Calves)/HN)	9	0.0594		9	2498.0		9	1446.1	
S (Sampling days)	3	0.3887	11.04***	3	4797.2	1.78	3	2651.0	8.01***
SH	6	0.0897	2.55*	6	990.8	0.37	6	1742.4	5.26**
SN	6	0.0808	2.30	6	826.5	0.31	6	1143.9	3.46*
SHN	12+	0.0272	0.77	12++	1405.3	0.52	12	1899.8	5.74***
Error (2) (SC/HN)	26+	0.0352		25++	2694.8		27	331.0	

* $P < 0.05$
** $P < 0.01$
*** $P < 0.001$
\+ 1.d.f. subtracted for 1 missing value estimated using Yates' method (117).
++ 2 d.f. subtracted for 2 missing values estimated using Yates' method (117).

APPENDIX 8: ANALYSIS OF VARIANCE: SERUM LACTIC DEHYDROGENASE AND INORGANIC PHOSPHORUS.

Source of Variation	L.D.H. (Replicate 1)			L.D.H. (Replicate 2)			Inorganic Phosphorus		
	d.f.	M.S.	F	d.f.	M.S.	F.	d.f.	M.S	F
H (H_2S levels)	2	14,906	1.12	2	309,030	2.00	2	0.1906	0.16
N (NH_3 levels)	2	25,356	1.90	2	312,260	2.02	2	0.2039	0.17
HN	4	23,195	1.74	4	129,600	0.84	4	1.9081	1.60
Error (1) (C(Calves)/HN)	9	13,317		9	154,340		9	1.1911	
S (Sampling days)	3	45,068	14.51***	3	82,535	4.84**	3	1.2083	6.00***
SH	6	4,038	1.30	6	20,444	1.20	6	0.7972	3.96**
SN	6	4,429	1.43	6	60,742	3.56**	6	0.1889	0.94
SHN	12	4,951	1.59	12	14,358	0.84	12	0.4469	2.22
Error (2) (SC/HN)	26+	3,106		27	17,065		26+	0.2015	

** $P < 0.01$
*** $P < 0.001$
+ 1 d.f. subtracted for 1 missing value estimated using Yates' method (117).

APPENDIX 9: ANALYSIS OF VARIANCE AND TABLE OF MEANS: SERUM ALKALINE PHOSPHATASE AND CALCIUM.

Source of Variation	Alkaline Phosphatase			Calcium		
	d.f.	M.S.	F	d.f.	M.S.	F
H (H_2S levels)	2	4148.8	0.68	2	0.3339	1.02
N (NH_3 levels)	2	19.0	0.00	2	0.0210	0.06
HN	4	1240.8	0.20	4	0.2851	0.87
Error (1) (C(Calves)/HN)	9	6112.3		9	0.3264	
S (Sampling days)	3	905.4	1.67	3	0.0861	1.45
SH	6	333.8	0.62	6	0.1300	2.19
SN	6	326.8	0.60	6	0.1104	1.86
SHN	12	462.9	0.85	12	0.0935	1.58
Error (2) (SC/HN)	26+	542.4		26+	0.0593	

+ 1 d.f. subtracted for 1 missing value estimated using Yates' method (117).

APPENDIX 9: Continued.....SAMPLE MEANS

Treatment H_2S	Treatment NH_3	Alkaline Phosphate (mIU/ml) Pre	Gas Day-2	Gas Day-7	Post Day-7	Calcium (mg/100 ml) Pre	Gas Day-2	Gas Day-7	Post Day-7
0	0	79.0	97.0	86.5	94.5	9.50	9.50	9.30	9.35
0	1	88.0	91.0	87.5	75.5	9.70	9.75	9.90	9.55
0	2	99.5	110.5	90.0	86.5	9.30	9.30	9.25	9.40
1	0	87.0	107.5	101.5	93.5	9.15	9.45	9.35	9.30
1	1	140.0	133.0	106.5	117.0	9.20	9.35	9.00	9.45
1	2	114.5	96.0	89.0	144.5	9.00	9.30	9.10	9.75
2	0	146.5	126.5	119.5	114.0	9.30	9.10	9.75	9.60
2	1	96.5	142.0	97.0	97.5	9.00	9.70	9.75	9.00
2	2	124.5	117.5	95.0	104.0	9.70	9.60	9.60	9.80
		S.E.M. = 16.5				S.E.M. = 0.17			
0		88.8	99.5	88.0	85.5	9.50	9.52	9.48	9.43
1		113.8	112.2	99.0	118.3	9.12	9.37	9.15	9.50
2		122.5	128.7	103.8	105.2	9.33	9.47	9.70	9.47
		S.E.M. = 9.5				S.E.M. = 0.10			
	0	104.2	110.3	102.5	100.7	9.32	9.35	9.47	9.42
	1	108.2	122.0	97.0	96.7	9.30	9.60	9.55	9.33
	2	112.8	108.0	91.3	111.7	9.33	9.40	9.32	9.65
		S.E.M. = 9.5				S.E.M. = 0.10			
		108.4	113.4	96.9	103.0	9.32	9.45	9.44	9.47
		S.E.M. = 5.5				S.E.M. 0.06			

APPENDIX 10: ANALYSIS OF VARIANCE AND TABLE OF MEANS: SERUM TOTAL PROTEIN AND ALBUMIN.

Source of Variation	Total Protein			Albumin		
	d.f.	M.S.	F	d.f.	M.S.	F
H (H_2S levels)	2	0.1388	0.28	2	0.0956	1.36
N (NH_3 levels)	2	0.0912	0.18	2	0.0018	0.03
HN	4	0.1381	0.28	4	0.1006	1.43
Error (1) (C(calves)/HN)	9	0.5015		9	0.0704	
S (Sampling days)	3	0.0716	0.74	3	0.0287	1.82
SH	6	0.0847	0.87	6	0.0268	1.70
SN	6	0.1144	1.17	6	0.0098	0.62
SHN	12	0.1277	1.31	12	0.0188	1.19
Error (2) (SC/HN)	26+	0.0974		26+	0.0158	

+ 1 d.f. subtracted for 1 missing value estimated using Yates' method (117).

APPENDIX 10: Continued - SAMPLE MEANS

Treatment H_2S	Treatment NH_3	Total Protein (g/100 ml) Pre	Gas Day-2	Gas Day-7	Post Day-7	Albumin (g/100 ml) Pre	Gas Day-2	Gas Day-7	Post Day-7
0	0	7.05	7.05	7.00	6.95	3.30	3.30	3.25	3.30
0	1	7.00	6.95	7.05	7.15	3.55	3.50	3.45	3.40
0	2	6.90	6.75	6.90	6.40	3.50	3.50	3.50	3.40
1	0	6.85	7.05	6.90	6.85	3.40	3.40	3.35	3.40
1	1	7.20	7.05	6.90	7.45	3.05	3.15	3.25	3.35
1	2	6.85	7.25	7.25	7.20	3.20	3.25	3.45	3.30
2	0	6.35	7.40	7.20	6.75	3.15	3.45	3.35	3.35
2	1	6.80	6.90	7.30	6.85	3.25	3.45	3.60	3.20
2	2	7.25	6.95	6.75	6.80	3.20	3.20	3.25	3.30
		S.E.M. = 0.22				S.E.M. = 0.09			
0		6.98	6.92	6.98	6.83	3.45	3.43	3.40	3.37
1		6.97	7.12	7.02	7.17	3.22	3.27	3.35	3.35
2		6.80	7.08	7.08	6.80	3.20	3.37	3.40	3.28
		S.E.M. = 0.13				S.E.M. = 0.05			
	0	6.75	7.17	7.03	6.85	3.28	3.38	3.32	3.35
	1	7.00	6.97	7.08	7.15	3.28	3.37	3.43	3.32
	2	7.00	6.98	6.97	6.80	3.30	3.32	3.40	3.33
		S.E.M. = 0.13				S.E.M. = 0.05			
		6.92	7.04	7.03	6.93	3.29	3.36	3.38	3.33
		S.E.M. = 0.07				S.E.M. = 0.03			

APPENDIX 11: ANALYSIS OF VARIANCE AND TABLE OF MEANS: SERUM GLUCOSE AND CHOLESTEROL.

Source of Variation	Glucose			Cholesterol		
	d.f.	M.S.	F	d.f.	M.S.	F
H (H_2S levels)	2	44.04	0.25	2	7.7	0.01
N (NH_3 levels)	2	153.29	0.87	2	1153.3	2.10
HN	4	227.71	1.29	4	362.5	0.66
Error (1) (C/HN)	9	176.29		9	548.1	
S (Sampling days)	3	159.94	5.13**	3	222.6	1.07
SH	6	14.80	0.47	6	122.6	0.59
SN	6	74.55	2.39	6	247.4	1.19
SHN	12	46.08	1.48	12	90.0	0.43
Error (2) (SC/HN)	26+	31.19		26+	207.6	

** $P < 0.01$

+ 1 d.f. subtracted for 1 missing value estimated using Yates' method (117).

APPENDIX 11: Continued.....SAMPLE MEANS

Treatment H_2S	Treatment NH_3	Glucose (mg/100 ml) Pre	Gas Day-2	Gas Day-7	Post Day-7	Cholesterol (mg/100 ml) Pre	Gas Day-2	Gas Day-7	Post Day-7
0	0	90.0	90.0	92.5	82.5	80.0	83.5	62.0	72.5
0	1	90.5	82.0	88.5	97.5	82.5	75.0	63.5	74.5
0	2	77.0	73.5	85.0	77.0	80.0	69.5	83.5	80.0
1	0	78.5	76.0	86.5	85.5	61.5	60.5	54.0	65.5
1	1	83.0	83.5	79.0	88.5	84.5	86.0	63.5	83.5
1	2	76.0	83.5	91.5	82.0	84.5	84.5	82.0	84.5
2	0	70.0	80.0	80.5	79.0	76.5	52.5	77.5	78.5
2	1	84.0	83.0	92.0	93.0	69.5	78.0	56.5	75.5
2	2	91.0	79.0	93.5	84.0	84.5	73.5	96.0	87.5
		S.E.M. = 4.0				S.E.M. = 10.2			
0		85.8	81.8	88.7	85.7	80.8	76.0	69.7	75.7
1		79.2	81.0	85.7	85.3	76.8	77.0	66.5	77.8
2		81.7	80.7	88.7	85.3	76.8	68.0	76.7	80.5
		S.E.M. = 2.3				S.E.M. = 5.9			
	0	79.5	82.0	86.5	82.3	72.7	65.5	64.5	72.2
	1	85.8	82.8	86.5	93.0	78.8	79.7	61.2	77.8
	2	81.3	78.7	90.0	81.0	83.0	75.8	87.2	84.0
		S.E.M. = 2.3				S.E.M. = 5.9			
		82.2	81.2	87.7	85.4	78.2	73.7	70.9	78.0
		S.E.M. = 1.3				S.E.M. = 3.4			

APPENDIX 12: ANALYSIS OF VARIANCE: RED BLOOD CELL COUNT, HEMATOCRIT, AND HEMOGLOBIN.

Source of Variation	Red Cell Count			Hematocrit			Hemoglobin		
	d.f.	M.S.	F	d.f.	M.S	F	d.f.	M.S.	F
H (H_2S levels)	2	1.002	0.42	2	18.042	1.21	2	1.4593	0.59
N (NH_3 levels)	2	2.624	1.09	2	21.375	1.44	2	0.5570	0.22
HN	4	9.906	4.12*	4	16.854	1.33	4	1.0595	0.43
Error (1) (C(Calves)/HN	9	2.405		9	14.875		9	2.4847	
S (Sampling days)	3	15.981	18.94***	3	35.384	4.13*	3	2.2820	4.92**
SH	6	1.409	1.67	6	11.523	1.35	6	0.5652	1.22
SN	6	0.464	0.55	6	7.968	0.93	6	0.3994	0.86
SHN	12+	1.457	1.73	12++	12.336	1.44	12++	0.9049	1.95
Error (2) (SC/HN)	25+	0.844		26++	8.563		26++	0.4641	

* P < 0.05
** P < 0.01
*** P < 0.001
\+ 2 d.f. subtracted for 2 missing values estimated using Yates' method (117).
++ 1 d.f. subtracted for 1 missing value estimated using Yates' method (117).

APPENDIX 13: ANALYSIS OF VARIANCE: TOTAL WHITE CELL COUNT AND PERCENTAGE DISTRIBUTION OF NEUTROPHILS, LYMPHOCYTES, AND MONOCYTES.

Source of Variation	Total White Cell Count			Neutrophil Percentage		
	d.f.	M.S.	F	d.f.	M.S.	F
H (H_2S levels)	2	1.8912×10^7	0.87	2	123.17	1.36
N (NH_3 levels)	2	3.9017×10^6	0.18	2	75.38	0.83
HN	4	2.9564×10^7	1.36	4	242.23	2.67
Error (1) (C(Calves)/HN)	9	2.1801×10^7		9	90.83	
S(Sampling days)	3	2.2210×10^7	2.27	3	173.06	1.64
SH	6	7.2245×10^6	0.74	6	35.33	0.33
SN	6	3.6583×10^6	0.37	6	29.43	0.28
SHN	12+	7.8439×10^6	0.80	12+	83.73	0.79
Error (2) (SC/HN)	26+	9.7842×10^6		26+	105.52	

Source of Variation	Lymphocytes			Monocytes		
	d.f.	M.S.	F	d.f.	M.S.	F
H (H_2S levels)	2	60.54	0.77	2	14.22	5.33*
N (NH_3 levels)	2	46.50	0.59	2	6.68	2.50
NH	4	231.35	2.93	4	1.70	0.64
Error (1) (C(Calves)/HN)	9	78.85		9	2.67	
S (Sampling days)	3	242.09	2.74	3	8.07	3.56*
SH	6	55.39	0.63	6	1.63	0.72
SN	6	22.24	0.25	6	2.31	1.02
SHN	12+	97.48	1.10	12+	5.30	2.33*
Error (2) (SC/HN)	26+	88.34		26+	2.27	

* $P \leq 0.05$ + 1 d.f. subtracted for 1 missing value estimated using Yates' method (117).

APPENDIX 14: ANALYSIS OF VARIANCE: BLOOD GASES AND HYDROGEN ION CONCENTRATION.

	Blood pO_2			Blood pCO_2			Blood pH		
Source of Variation	d.f.	M.S.	F	d.f.	M.S.	F	d.f	M.S.	F
H (H_2S levels)	2	5.16	1.37	2	22.66	2.50	2	0.000939	0.23
N (NH_3 levels)	2	26.21	6.97*	2	15.96	1.76	2	0.001217	0.29
HN	4	4.38	1.17	4	10.63	1.17	4	0.003456	0.83
Error (1) (C(Calves)/HN)	9	3.76		9	9.08		9	0.004143	
S (Sampling days)	2	314.32	21.68***	2	260.05	58.40***	2	0.002817	0.94
SH	4	8.10	0.56	4	4.24	0.95	4	0.005381	1.80
SN	4	0.94	0.06	4	6.88	1.55	4	0.001550	0.52
SHN	8	19.01	1.31	8	3.77	0.85	8	0.002814	0.94
Error (2) (SC/HN)	18	14.50		18	4.45		18	0.002982	

* $P < 0.05$
*** $P < 0.001$